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(54) Title: ANTIBODIES AGAINST CARBOXIC ANHYDRASE IX (CA IX) TUMOR ANTIGEN

Cell	Single Cell	Vgamma/D/J	FR1	CDR1	FR2	CDR2
–	–	[redacted]	QVQLQESGPGLVKPSETLSLTCTVSGGSIS	SYYWS	WIRQPPGKGLEWIG	YIYYSGSTNYNPSLKS
43G10	80	VH4-59/D3-3/JH6b	——————S——————	————	————————————	————————————
44D2	141		————————————	————	————————————	————————————
41D1	174		————————————	————	————————————	——F—————————
–	–	[redacted]	QVQLQESGPGLVKPSQTLSLTCTVSGGSIS	SGGYYWS	WIRQHPGKGLEWIG	YIYYSGSTYYNPSLKS
45C9	23	VH4-30.1/D3-9/JH5b	————————————	————	————————————	————————————
–	–	[redacted]	EVQLVESGGGLVKPGGSLRLSCAASGFTFS	SYSMN	WVRQAPGKGLEWVS	SISSSSSYIYYADSVKG
49C8	51	VH3-21/D5-18/JH4b	——————F—————————	-FR———	———————E————	——T—————————
–	–	[redacted]	QVQLQESGPGLVKPSETLSLTCTVSGGSIS	SYYWS	WIRQPAGKGLEWIG	RIYTSGSTNYNPSLKS
42E4	109	VH4-04/D1-20/JH6b	————————————	————	————————————	————————————

Cell	Single Cell	Vgamma/D/J	FR3	CDR3	FR4	
–	–	[redacted]	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR		WGQGTTVTVSS	SEQ ID NO: 70
43G10	80	VH4-59/D3-3/JH6b	————————————	DTRTIFGVVSGNDV	————————	SEQ ID NO: 71
44D2	141		————N-H—————	DTRTIFGVVSGNDV	————————	SEQ ID NO: 72
41D1	174		————————————	DTRTIFGVVSGNDV	————————	SEQ ID NO: 73
–	–	[redacted]	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR		WGQGTLVTVSS	SEQ ID NO: 74
45C9	23	VH4-30.1/D3-9/JH5b	————————————	ENYDILTGFNWFDP	————————	SEQ ID NO: 75
–	–	[redacted]	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR		WGQGTLVTVSS	SEQ ID NO: 76
49C8	51	VH3-21/D5-18/JH4b	————————————	FTAMALDY	————————	SEQ ID NO: 77
–	–	[redacted]	RVTMSVDTSKNQFSLKLSSVTAADTAVYYCAR		WGQGTTVTVSS	SEQ ID NO: 78
42E4	109	VH4-04/D1-20/JH6b	————————————	LITGPYGMDV	————————	SEQ ID NO: 79

(57) Abstract: The present invention relates generally to the generation and characterization of anti-CA IX monoclonal antibodies. The invention further relates to the use of such anti-CA IX antibodies in the diagnosis and treatment of disorders associated with increased activity of CA IX, in particular, tumors such as colorectal cancer, renal cell carcinoma (RCC), cervical and other cancers of epithelial origin.

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ANTIBODIES AGAINST CARBOXIC ANHYDRASE IX (CA IX) TUMOR ANTIGEN

Background of the Invention

Field of the Invention

Embodiments of the present invention concern antibodies binding carbonic anhydrase IX (CA IX) tumor antigen as well as methods and means for making and using such antibodies.

Description of the Related Art

The therapeutic utility of monoclonal antibodies (mAbs) (G. Kohler and C. Milstein, *Nature* 256:495-497 (1975)) is being realized. Monoclonal antibodies have now been approved as therapies in transplantation, cancer, infectious disease, cardiovascular disease and inflammation. Different isotypes have different effector functions. Such differences in function are reflected in distinct 3-dimensional structures for the various immunoglobulin isotypes (P.M. Alzari et al., *Annual Rev. Immunol.*, 6:555-580 (1988)).

Because mice are convenient for immunization and recognize most human antigens as foreign, mAbs against human targets with therapeutic potential have typically been of murine origin. However, murine mAbs have inherent disadvantages as human therapeutics. They require more frequent dosing as mAbs have a shorter circulating half-life in humans than human antibodies. More critically, the repeated administration of murine antibodies to the human immune system causes the human immune system to respond by recognizing the mouse protein as a foreign and generating a human anti-mouse antibody (HAMA) response. Such a HAMA response may result in allergic reaction and the rapid clearing of the murine antibody from the system thereby rendering the treatment by murine antibody useless. To avoid such affects, attempts to create human immune systems within mice have been attempted.

Initial attempts hoped to create transgenic mice capable of responding to antigens with antibodies having human sequences (See Bruggemann et al., *Proc. Nat'l. Acad. Sci. USA* 86:6709-6713 (1989)), but were limited by the amount of DNA that could be stably maintained by available cloning vehicles. The use of yeast artificial chromosome (YAC) cloning vectors led the way to introducing large germline fragments of human Ig locus into transgenic mammals. Essentially a majority of the human V, D, and J region genes arranged with the same spacing found in the human genome and the human constant regions were introduced into mice using YACs. One such transgenic mouse strain is known as XenoMouse(r) mice and is commercially available from Abgenix, Inc. (Fremont CA).

XenoMouse mice are strains of mice that have inactivated mouse IgH and IgK loci and is transgenic for functional megabase-sized human IgH and IgK transgenes. Further, XenoMouse mice are transgenic mice capable of producing high affinity, fully human antibodies of the desired isotype (i.e., IgG1) in response to immunization with virtually any desired antigen. Such a mAbs

can be used to direct complement dependent cytotoxicity or antibody-dependent cytotoxicity to a target cell.

Because CA IX is a biomarker found to be overexpressed in most cervical carcinomas (Brewer et al., *Gynecol. Oncol.*, 63(3): 337-44 (1996)) and in some tumors, particularly renal cell carcinoma (Beasley et al., *Cancer Res.*, 61(13):5262-7 (2001) esophageal cancers (Kaluz et al., *J. Biol. Chem.*, 274946):32588-32595 (1999)), and breast cancer, the role of CA IX in tumor cell progression and growth is of intense interest. According to Chia et al., *J. Clin. Oncol.*, 19(16):3660-8 (2001), CA IX, also referred to as MN, may have a role as a marker of hypoxia in carcinomas. Further, overexpression of CA IX may help to maintain the intracellular pH (Beasley et al., *Cancer Res.* 61(13):5262-7 (2001), giving tumor cells a survival advantage and enhancing resistance to radiotherapy and chemotherapy. Accordingly, CA IX may be an ideal potential target for therapy against such tumors.

Monoclonal antibodies specific for CA IX have been generated (Zavada et al., *Br. J. Cancer*, 82(11):1808-13 (2000)). These antibodies were specifically directed to the adhesion domain and affected the ability of CA IX to attach to tumor cells. The adhesion domain is located in the proteoglycan domain and contains a sixfold tandem repeat of the six amino acid sequence GEEDLP (Brewer et al., *Gynecol Oncol.*, 63(3); 337-44 (1996). From a phage display library of random heptapeptides, several heptapeptides with the ability to compete for the adhesion epitope on CA IX and inhibit adhesion of cells to CA IX have been identified.

It is believed that the first anti-MN antibody was the G250 antibody generated by Oosterwijk et al. (Am J Pathol 1986 May;123(2):301-9; Oosterwijk et al. Int J Cancer 1986 Oct 15;38(4):489-94) which was an antibody that bound to an antigen that was expressed preferentially in renal cell carcinomas. The G250 antibody was subsequently identified to bind to the MN antigen (Uemura et al. Br J Cancer 1999 Oct;81(4):741-6).

Although monoclonal antibodies have been identified that specifically bind to CA IX, antibodies that specifically inhibit its biological activities, such as cell proliferation and survival which may be essential to tumor progression are needed. The ability to affect the growth and/or survival of tumor cells expressing CA IX on the cell surface may prove to be a treatment having widespread application to many patients afflicted with tumors.

Summary of the Invention

Embodiments of the present invention are based on the development of monoclonal antibodies that were found to bind CA IX and affect CA IX function.

In one aspect, the invention provides an anti-human CA IX monoclonal antibody which binds to and neutralizes a biological activity of at least human CA IX. The antibody can significantly reduce or eliminate a biological activity of the human CA IX in question.

The biologic activity of the subject human CA IX is likely to be important for cell proliferation or survival under conditions of stress.

In one embodiment, the invention provides an isolated monoclonal antibody comprising a heavy chain amino acid, having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 24, 25, 26, 27, 28, 29, 30, 71, 72, 73, 75, 77, 79, 90, 92, 94, 96, 98, 100, 102, 125, 128, 130, 133, 136, 137, 142, 146, 150, 153, 169, 171, 172 and 246 and wherein said monoclonal antibody specifically binds CA IX. The antibody is preferably a fully human antibody. In a further embodiment, the antibody further comprises a light chain amino acid having an amino acid sequence selected from the group consisting of SEQ ID NOs: 32, 33, 34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 60, 61, 62, 63, 64, 66, 67, 68, 69, 81, 82, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 162, 168, 175, 177, 179 and 245. The monoclonal antibody may further be associated with a therapeutically acceptable carrier or may be conjugated to a therapeutic or cytotoxic agent whereing the further therapeutic agent is a toxin, such as a catalytic toxin, ricin, Pseudomonas exotoxin, or the drug-like toxin, maytansinoid, auristatinE or geldanamycin, or a radioisotope. Preferably, such an antibody may be used for treatment of diseases, such as tumors.

In another aspect, the invention provides a method of inhibiting cell proliferation associated with the expression of CA IX tumor antigen comprising treating cells expressing CA IX with an effective amount of an CA IX monoclonal antibody.

In another aspect, the invention provides a method for treatment of a disease associated with the expression of CA IX in a patient, comprising administering to the patient an effective amount of an CA IX monoclonal antibody. The patient is preferably a human mammalian patient and the disease is preferably a tumor that is selected from the group consisting of colorectal neoplasms, colorectal tumors, renal cell carcinoma (RCC), cervical carcinoma, cervical intraepithelial squamous and glandular neoplasia, esophageal tumors, and breast cancer.

Also provided is an isolated nucleic acid molecule encoding any of the antibodies described herein, a vector comprising the isolated nucleic acid molecule, a host cell transformed with the nucleic acid molecule, and a method of producing the antibody comprising culturing the host cell under conditions wherein the nucleic acid molecule is expressed to produce the antibody and optionally recovering the antibody from the host cell. The antibody may be of the IgG class. The isolated nucleic acid molecule preferably comprises a nucleotide sequence encoding a heavy chain variable domain of a monoclonal antibody, wherein said nucleotide sequence is selected from the group consisting of SEQ ID NOs: 104, 106, 108, 110, 112, 114, 116, 118, 170, 172, 174, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211 and 212, or a nucleotide sequence encoding a light chain variable domain of a monoclonal antibody, wherein said nucleotide sequence is selected from the group consisting of SEQ ID NOs: 105, 107, 109, 111, 113, 115, 117, 119, 176, 178, 180, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243 and 244.

In yet another aspect, the invention provides an antibody of the invention linked to a radioisotope.

Brief Description of the Drawings

Figure 1 shows the alignment of the amino acid sequences encoding for the heavy chain variable domain regions of the immunoglobulins directed against CA IX, AB-MN-21.17.1_HC (SEQ ID NO: 2), AB-MN-22.19_HC (SEQ ID NO: 3), AB-MN-22.29_HC (SEQ ID NO: 4), AB-MN-22.3_HC (SEQ ID NO: 5) and AB-MN-22.5_HC (SEQ ID NO: 6), with germline Vgamma/D/J sequence of VH4-4 (SEQ ID NO: 1). The CDRs, CDR1, CDR2, CDR3 in the immunoglobulins are shown under the solid horizontal lines. "AB-MN-21." antibodies are generated in IgG1 mice and "AB-MN-22." antibodies are generated in IgG2 mice. The letters in bold represent conservative and non-conservative amino acid changes.

Figure 2 shows a dendrogram representing the hierarchy of the anti-CA IX immunoglobulins, AB-MN-21.17.1_HC, AB-MN-22.19_HC, AB-MN-22.29_HC , AB-MN-22.3_HC and AB-MN-22.5_HC, based on their similarity with the variable heavy chain region,VH4-4.

Figure 3A-B shows the alignment of the amino acid sequences encoding for the heavy chain variable domain regions of the immunoglobulins directed against CA IX, AB-MN-21.2.1_HC (SEQ ID NO: 8), AB-MN-21.5.2_HC (SEQ ID NO: 9), AB-MN-21.6.1_HC (SEQ ID NO: 10), AB-MN-21.7.1_HC (SEQ ID NO: 11), AB-MN-21.9.1_HC (SEQ ID NO: 12), AB-MN-22.11_HC (SEQ ID NO: 13), AB-MN-22.15_HC (SEQ ID NO: 14), AB-MN-22.16_HC (SEQ ID NO: 15), AB-MN-22.18_HC (SEQ ID NO: 16), AB-MN-22.21_HC (SEQ ID NO: 17), AB-MN-22.23_HC (SEQ ID NO: 18) and AB-MN-22.9_HC (SEQ ID NO: 19) with germline Vgamma/D/J sequence of VH4-31 (SEQ ID NO: 7). The CDRs, CDR1, CDR2, CDR3 in the immunoglobulins are shown under the solid horizontal lines. "AB-MN-21." antibodies are generated in IgG1 mice and "AB-MN-22." antibodies are generated in IgG2 mice. The letters in bold represent conservative and non-conservative amino acid changes.

Figure 4 shows a dendrogram representing the hierarchy of the anti-CA IX immunoglobulins, AB-MN-21.2.1_HC, AB-MN-21.5.1_HC, AB-MN-21.6.1_HC , AB-MN-21.7.1_HC, AB-MN-21.9.1_HC, AB-MN-22.11_HC, AB-MN-22.15_HC, AB-MN-22.16_HC, AB-MN-22.18_HC, AB-MN-22.21_HC, AB-MN-22.23_HC and AB-MN-22.9_HC based on their similarity with the variable heavy chain region,VH4-31.

Figure 5 shows the alignment of the amino acid sequences encoding for the heavy chain variable domain regions of the immunoglobulins directed against CA IX, AB-MN-21.10.1_HC (SEQ ID NO: 21) and AB-MN-22.25_HC (SEQ ID NO: 22) with germline Vgamma/D/J sequence of VH4-39 (SEQ ID NO: 20). The CDRs, CDR1, CDR2, CDR3 in the immunoglobulins are shown under the solid horizontal lines. "AB-MN-21." antibodies are generated in IgG1 mice and "AB-

MN-22." antibodies are generated in IgG2 mice. The letters in bold represent conservative and non-conservative amino acid changes.

Figure 6 shows the alignment of the amino acid sequences encoding for the heavy chain variable domain regions of the immunoglobulins directed against CA IX, AB-MN-21.1.1_HC (SEQ ID NO: 24), AB-MN-21.14.1_HC (SEQ ID NO: 25), AB-MN-22.12_HC (SEQ ID NO: 26), AB-MN-22.17_HC (SEQ ID NO: 27), AB-MN-22.26_HC (SEQ ID NO: 28), AB-MN-22.27_HC (SEQ ID NO: 29) and AB-MN-22.8.1_HC (SEQ ID NO: 30) with germline Vgamma/D/J sequence of VH4-59 (SEQ ID NO: 23). The CDRs, CDR1, CDR2, CDR3 in the immunoglobulins are shown under the solid horizontal lines. "AB-MN-21." antibodies are generated in IgG1 mice and "AB-MN-22." antibodies are generated in IgG2 mice. The letters in bold represent conservative and non-conservative amino acid changes.

Figure 7 shows a dendrogram representing the hierarchy of the anti-CA IX immunoglobulins, AB-MN-21.1.1_HC, AB-MN-21.14.1_HC, AB-MN-22.12_HC, AB-MN-22.17_HC, AB-MN-22.26_HC, AB-MN-22.27_HC and AB-MN-22.8.1_HC based on their similarity with the variable heavy chain region of VH4-59.

Figure 8 shows the alignment of the amino acid sequences encoding for the light chain variable domain regions of the immunoglobulins directed against CA IX, AB-MN-21.5.2_LC (SEQ ID NO: 32), AB-MN-21.6.1_LC (SEQ ID NO: 33) and AB-MN-22.7_LC (SEQ ID NO: 34) with germline V_{Kappa}/J sequence of VK-A19 (SEQ ID NO: 31). The CDRs, CDR1, CDR2, CDR3 in the immunoglobulins are shown under the solid horizontal lines. "AB-MN-21." antibodies are generated in IgG1 mice and "AB-MN-22." antibodies are generated in IgG2 mice. The letters in bold represent conservative and non-conservative amino acid changes.

Figure 9 shows a dendrogram representing the hierarchy of the anti-CA IX immunoglobulins, AB-MN-21.5.2_LC, AB-MN-21.6.1_LC and AB-MN-22.7_LC based on their similarity with the variable light chain region of VK-A19.

Figure 10 shows the alignment of the amino acid sequences encoding for the light chain variable domain regions of the immunoglobulins directed against CA IX, AB-MN-22.8.1_LC (SEQ ID NO: 36), AB-MN-21.17.1_LC (SEQ ID NO: 37), AB-MN-21.8.1_LC (SEQ ID NO: 38), AB-MN-22.11_LC (SEQ ID NO: 39), AB-MN-22.19_LC (SEQ ID NO: 40), AB-MN-22.26_LC (SEQ ID NO: 41), AB-MN-22.27_LC (SEQ ID NO: 42), AB-MN-22.3_LC (SEQ ID NO: 43), AB-MN-22.4_LC (SEQ ID NO: 44), AB-MN-22.5_LC (SEQ ID NO: 45) and AB-MN-22.9_LC (SEQ ID NO: 46) with germline V_{Kappa}/J sequence of VK-A27 (SEQ ID NO: 35). The CDRs, CDR1, CDR2, CDR3 in the immunoglobulins are shown under the solid horizontal lines. "AB-MN-21." antibodies are generated in IgG1 mice and "AB-MN-22." antibodies are generated in IgG2 mice. The letters in bold represent conservative and non-conservative amino acid changes.

Figure 11 shows a dendrogram representing the hierarchy of the anti-CA IX immunoglobulins, AB-MN-22.8.1_LC, AB-MN-21.17.1_LC, AB-MN-21.8.1_LC, AB-MN-

22.11_LC, AB-MN-22.19_LC, AB-MN-22.26_LC, AB-MN-22.27_LC, AB-MN-22.3_LC, AB-MN-22.4_LC, AB-MN-22.5_LC and AB-MN-22.9_LC based on their similarity with the variable light chain region of VK-A27.

Figure 12 shows the alignment of the amino acid sequences encoding for the light chain variable domain regions of the immunoglobulins directed against CA IX, AB-MN-21.2.1_LC (SEQ ID NO: 48), AB-MN-21.7.1_LC (SEQ ID NO: 49), AB-MN-22.10_LC (SEQ ID NO: 50), AB-MN-22.14_LC (SEQ ID NO: 51), AB-MN-22.18_LC (SEQ ID NO: 52), AB-MN-22.20_LC (SEQ ID NO: 53), AB-MN-22.21_LC (SEQ ID NO: 54), AB-MN-22.24_LC (SEQ ID NO: 55), AB-MN-22.25_LC (SEQ ID NO: 56), AB-MN-22.28.1_LC (SEQ ID NO: 57) and AB-MN-22.30_LC (SEQ ID NO: 58) with germline V_{Kappa}/J sequence of VK-A30 (SEQ ID NO: 47). The CDRs, CDR1, CDR2, CDR3 in the immunoglobulins are shown under the solid horizontal lines. "AB-MN-21." antibodies are generated in IgG1 mice and "AB-MN-22." antibodies are generated in IgG2 mice. The letters in bold represent conservative and non-conservative amino acid changes.

Figure 13 shows a dendrogram representing the hierarchy of the anti-CA IX immunoglobulins, AB-MN-21.2.1_LC, AB-MN-21.7.1_LC, AB-MN-22.10_LC, AB-MN-22.14_LC, AB-MN-22.18_LC, AB-MN-22.20_LC, AB-MN-22.21_LC, AB-MN-22.24_LC, AB-MN-22.25_LC, AB-MN-22.28.1_LC and AB-MN-22.30_LC based on their similarity with the variable light chain region of VK-A30.

Figure 14 shows the alignment of the amino acid sequences encoding for the light chain variable domain regions of the immunoglobulins directed against CA IX, AB-MN-21.1.1_LC (SEQ ID NO: 60), AB-MN-21.14.1_LC (SEQ ID NO: 61), AB-MN-22.12_LC (SEQ ID NO: 62), AB-MN-22.17_LC (SEQ ID NO: 63) and AB-MN-22.29_LC (SEQ ID NO: 64) with germline V_{Kappa}/J sequence of VK-L5 (SEQ ID NO: 59). The CDRs, CDR1, CDR2, CDR3 in the immunoglobulins are shown under the solid horizontal lines. "AB-MN-21." antibodies are generated in IgG1 mice and "AB-MN-22." antibodies are generated in IgG2 mice. The letters in bold represent conservative and non-conservative amino acid changes.

Figure 15 shows a dendrogram representing the hierarchy of the anti-CA IX immunoglobulins, AB-MN-21.1.1_LC, AB-MN-21.14.1_LC, AB-MN-22.12_LC, AB-MN-22.17_LC and AB-MN-22.29_LC based on their similarity with the variable light chain region of VK-L5.

Figure 16 shows the alignment of the amino acid sequences encoding for the light chain variable domain regions of the immunoglobulins directed against CA IX, AB-MN-21.9.1_LC (SEQ ID NO: 66), AB-MN-22.15_LC (SEQ ID NO: 67), AB-MN-22.16_LC (SEQ ID NO: 68) and AB-MN-22.23_LC (SEQ ID NO: 69) with germline V_{Kappa}/J sequence of VK-O12 (SEQ ID NO: 65). The CDRs, CDR1, CDR2, CDR3 in the immunoglobulins are shown under the solid horizontal lines. "AB-MN-21." antibodies are generated in IgG1 mice and "AB-MN-22." antibodies are

generated in IgG2 mice. The letters in bold represent conservative and non-conservative amino acid changes.

Figure 17 shows a dendrogram representing the hierarchy of the anti-CA IX immunoglobulins, AB-MN-21.9.1_LC, AB-MN-22.15_LC, AB-MN-22.16_LC and AB-MN-22.23_LC based on their similarity with the variable light chain region of VK-O12.

Figure 18A and 18B shows the alignment of the amino acid sequences encoding for the heavy chain variable domain regions of the immunoglobulins directed against CA IX, AB-MN-XG1-080 (SEQ ID NO: 71), AB-MN-XG1-141 (SEQ ID NO: 72), AB-MN-XG1-174 (SEQ ID NO: 73) with germline Vgamma/D/J sequence of VH4-59 (DP-71)/D3-JH4b (SEQ ID NO: 70), AB-MN-XG1-023 (SEQ ID NO: 75), germline Vgamma/D/J sequence of VH4-30.1 (DP-65)/D3-9/JH5b (SEQ ID NO: 74) AB-MN-XG1-051 (SEQ ID NO: 77) germline Vgamma/D/J sequence of VH3.21 (DP-77)/D5-18/JH4b (SEQ ID NO: 76), and AB-MN-XG1-109 (SEQ ID NO: 79) with germline Vgamma/D/J sequence of VH4.04 (VIV-4)/D1-20/JH6b (SEQ ID NO 78). The CDRs, CDR1, CDR2, CDR3 in the immunoglobulins are shown under respective column headings. These "AB-MN-XG1" antibodies are generated in IgG1 Xenomice.

Figure 19A and 19B shows the alignment of the amino acid sequences encoding for the light chain variable domain regions of the immunoglobulins directed against CA IX, AB-MN-XG1-080 (SEQ ID NO: 81), AB-MN-XG1-141 (SEQ ID NO: 82), AB-MN-XG1-174 (SEQ ID NO: 83) with germline V_{Kappa}/J sequence of A27/JK4 (SEQ ID NO: 80), AB-MN-XG1-023 (SEQ ID NO: 85), germline V_{Kappa}/J sequence of L19/JK1 (SEQ ID NO: 84) AB-MN-XG1-051 (SEQ ID NO: 87) germline V_{Kappa}/J sequence of A20/JK1 (SEQ ID NO: 86), and AB-MN-XG1-109 (SEQ ID NO: 89) with germline V_{Kappa}/J sequence of O2/JK3 (SEQ ID NO 88). The CDRs, CDR1, CDR2, CDR3 in the immunoglobulins are shown under respective column headings. These "AB-MN-XG1" antibodies are generated in IgG1 Xenomice.

Figure 20 represents immunofluorescence results showing that anti-CA IX antibodies, AB-MN-XG2-109, AB-MN-XG2-051 and AB-MN-XG2-023 bind to tumor cells while control antibodies against IL8, AB-hIL8-XG2142, do not bind to HT-29 tumor cells.

Figures 21A-C represent cell viability assay results showing that anti-CA IX antibodies, AB-MN-XG1-023 (MN23), AB-MN-XG1-080 (MN80), AB-MN-XG1-141 (MN141), AB-MN-XG1-174 (MN174) and AB-MN-XG1-051 (MN51) reduced the viability of HeLa and MDA 468 cells expressing endogenous CA IX antigen the affect of anti-CA IX antibodies on the viability of HeLa cell and MDA468 cells expressing endogenous CA IX.

Figure 22 represents immunofluorescences results showing that anti-CA IX antibodies, AB-MN-XG1-051 (MN51) and AB-MN-XG1-023 (MN23) were internalized into SKRC10 cells.

Figures 23A-C shows the induction of antibody-dependent cell-mediated cytotoxicity (ADCC) by anti-CA IX antibodies in HT-29 or SK-RC-52, cells from a renal cell carcinoma cell line, target cells.

Figures 24A,B represent cell based array (CBA) results that show the level of CA IX expression in a number of tumor cell lines. Human CA IX expression in melanoma, cervical and ovarian cancer cell lines are shown in the upper panel while human CA IX expression in pancrease, prostate and renal cancer cell lines are shown in the lower panel of the figure.

Figure 25 represents cell based array (CBA) results that show upregulation of CA IX expression in cells cultured in hypoxic conditions.

Figures 26A-B show the alignment of the amino acid sequences encoding for the heavy chain variable domain regions of the immunoglobulins directed against CA IX, AX014H21_8_1N1G2 (SEQ ID NO: 125) with germline Vgamma/D/J sequence of VH1-2/D7-27/JH6b (SEQ ID NO: 124), AX014H22_5N1G2 (SEQ ID NO: 6), AX014H22_19N1G2 (SEQ ID NO: 3) and AX014H22_3N1G2 (SEQ ID NO: 5) with germline Vgamma/D/J sequence of VH4-4/D3-3/JH6b (SEQ ID NO: 126), AX014H22_14N1G2 (SEQ ID NO: 128) with Vgamma/D/J sequence of VH4-61/D3-10/JH46 (SEQ ID NO: 127), AX014H22_28.1N1G (SEQ ID NO: 130) with Vgamma/D/J sequence of VH3-23/D1-26/JH6b (SEQ ID NO: 129), AX014H22_23N1G2 (SEQ ID NO: 18) and AX014h22_9N1G2 (SEQ ID NO: 19) with Vgamma/D/J sequence of VH4-31/D4-17/JH6b (SEQ ID NO: 131), AX014H22_7N1G2 (SEQ ID NO: 133) with Vgamma/D/J sequence of VH3-30/D3-10/JH6b (SEQ ID NO: 132), AX014H21_17_1N1G (SEQ ID NO: 2) and AX014H22_29N1G2 (SEQ ID NO: 4) with Vgamma/D/J sequence of VH4-4/D6-19/JH6b (SEQ ID NO: 134), AX014H22_10N1G2 (SEQ ID NO: 136) AND AX014H22_24N1G2 (SEQ ID NO: 137) with Vgamma/D/J sequence of VH6-1/D1-26/JH4b (SEQ ID NO: 135), AX014H21_5_2N1G2 (SEQ ID NO: 9) and AX014H21_6_1N1G2 (SEQ ID NO: 10) with Vgamma/D/J sequence of VH4-31/D3-10/JH4b (SEQ ID NO: 138), AX014H22_21N1G2 (SEQ ID NO: 17) with Vgamma/D/J sequence of VH4-31/D4-11/JH6b (SEQ ID NO: 139), AX014H22_16N1G2 (SEQ ID NO: 15) with VH4-31/D2-21/JH6b (SEQ ID NO: 140), AX014H22_4N1G2 (SEQ ID NO: 142) with Vgamma/D/J sequence of VH1-2/D1-26/JH4b (SEQ ID NO: 141). AX014H21_1_1N1G2 (SEQ ID NO: 24), AX014H22_27N1G2 (SEQ ID NO: 29), AX014H21_14_1N1G2 (SEQ ID NO: 25), AX014H22_17N1G2 (SEQ ID NO: 27) and AX014H22_26N1G2 (SEQ ID NO: 28) with Vgamma/D/J sequence of VH4-59/D3-9/JH4b (SEQ ID NO: 143). The CDRs, CDR1, CDR2, CDR3 and FR regions in the immunoglobulins are shown under the respective column headings. The "AX014H21" and "AX014H22" antibodies are generated in IgG1 and IgG2 Xenomice, respectively.

Figure 27A-B show the alignment of the amino acid sequences encoding for the heavy chain variable domain regions of the immunoglobulins directed against CA IX, AX014H22_11N1G2 (SEQ ID NO: 13) with germline Vgamma/D/J sequence of VH4-31/D5-24/JH2 (SEQ ID NO: 144), AX014H22_13_1N1G2 (SEQ ID NO: 146) with germline Vgamma/D/J sequence of VH3-48/JH6b (SEQ ID NO: 145), AX014H22_15N1G2 (SEQ ID NO: 14) with germline Vgamma/D/J sequence of VH4-31/D3-9/JH46 (SEQ ID NO: 147), AX014H22_25N1G2

(SEQ ID NO: 22) and AX014H21_10_1N1G2 (SEQ ID NO: 21) with germline Vgamma/D/J sequence of VH4-39/JH6b (SEQ ID NO: 148), AX014H22_30N1G2 (SEQ ID NO: 150) with germline Vgamma/D/J sequence of VH3-33/D3-10/JH6b (SEQ ID NO: 149), AX014H21_9_1N1G2 (SEQ ID NO: 12) with germline Vgamma/D/J sequence of VH4-31/D3-10/JH6b (SEQ ID NO: 151), AX014H22_18N1G2 (SEQ ID NO: 16), AX014H21_7_1N1G2 (SEQ ID NO: 11), AX014H22_20N1G2 (SEQ ID NO: 153) and AX014H21_2_1N1G2 (SEQ IDNO: 8) with germline Vgamma/D/J sequence of VH4-31/D3-9/JH6b (SEQ ID NO: 152), and AX014H22_8_1N1G2 (SEQ ID NO: 30) and AX014H22_12N1G2 (SEQ ID NO: 26) with germline Vgamma/D/J sequence of VH4-59/D6-13/JH6b (SEQ ID NO: 154). The CDRs, CDR1, CDR2, CDR3 and FR regions in the immunoglobulins are shown under the respective column headings. The "AX014H21" and "AX014H22" antibodies are generated in IgG1 and IgG2 Xenomice, respectively.

Figure 28A-B show the alignment of the amino acid sequences encoding for the light chain variable domain regions of the immunoglobulins directed against CA IX, AX014H22_15N1K (SEQ ID NO: 67), AX014H22_23N1K (SEQ ID NO: 69), AX014H21_9_1N1K (SEQ ID NO: 66) and AX014H22_16N1K (SEQ ID NO: 68) with germline Vkappa/J sequence of O12/JK4 (SEQ ID NO: 155), AX014H22_3N1K (SEQ ID NO: 43) with germline Vkappa/J sequence of A27/JK5 (SEQ ID NO: 156), AX014H21_6_1N1K (SEQ ID NO: 33) and AX014H21_5_2N1K (SEQ ID NO: 32) with germline Vkappa/J sequence of A3/JK4 (SEQ ID NO: 157), AX014H22_8_1N1K (SEQ ID NO: 36), AX014H22_27N1K (SEQ ID NO: 42), AX014H22_5N1K (SEQ ID NO: 45), AX014H21_17_1N1K (SEQ ID NO: 37), AX014H22_11N1K (SEQ ID NO: 39), AX014H21_8_1N1K (SEQ ID NO: 38), AX014H22_19N1K (SEQ ID NO: 40) and AX014H22_4N1K (SEQ ID NO: 44) with germline Vkappa/J sequence of A27/JK4 (SEQ ID NO: 80), AX014H22_30N1K (SEQ ID NO: 58), AX014H22_14N1K (SEQ ID NO: 51) and AX014H22_28_1N1K (SEQ ID NO: 57) with germline Vkappa/J sequence of A30/JK4 (SEQ ID NO: 158), AX014H21_1_1N1K (SEQ ID NO: 60), AX014H22_29N1K (SEQ ID NO: 64), AX014H21_14_1N1K (SEQ ID NO: 61) and AX014H22_17N1K (SEQ ID NO: 63) with germline Vkappa/J sequence of L5/JK5 (SEQ ID NO: 159), AX014H22_24N1K (SEQ ID NO: 55) and AX014H22_10N1K (SEQ ID NO: 50) with germline Vkappa/J sequence of A30/JK3 (SEQ ID NO: 160), AX014H21_10_1N1K (SEQ ID NO: 162) with germline Vkappa/J sequence of A1/JK4 (SEQ ID NO: 161) and AX014H22_9N1K (SEQ ID NO: 46) and AX014H22_26N1K (SEQ ID NO: 41) with germline Vkappa/J sequence of A27/JK2 (SEQ ID NO: 163). The CDRs, CDR1, CDR2, CDR3 and FR regions in the immunoglobulins are shown under the respective column headings. The "AX014H21" and "AX014H22" antibodies are generated in IgG1 and IgG2 Xenomice, respectively.

Figure 29A-B show the alignment of the amino acid sequences encoding for the light chain variable domain regions of the immunoglobulins directed against CA IX, AX014H22_12N1K (SEQ

ID NO: 62) with germline Vkappa/J sequence of L5/JK3 (SEQ ID NO: 164), AX014H22_7N1K (SEQ ID NO: 34) with germline Vkappa/J sequence of A3/JK2 (SEQ ID NO: 165), AX014H22_18N1K (SEQ ID NO: 52), AX014H21_7_1N1K (SEQ ID NO: 49), AX014H22_21N1K (SEQ ID NO: 54), AX014H22_20N1K (SEQ ID NO: 53), AX014H22_25N1K (SEQ ID NO: 56) AND AX014H21_2_1N1K (SEQ ID NO: 48) with germline Vkappa/Jsequence of A30/JK1 (SEQ ID NO: 166) and AX014H22_13_1N1K (SEQ ID NO: 168) with germline Vkappa/J sequence of A23/JK4 (SEQ ID NO: 167). The CDRs, CDR1, CDR2, CDR3 and FR regions in the immunoglobulins are shown under the respective column headings. The "AX014H21" and "AX014H22" antibodies are generated in IgG1 and IgG2 Xenomice, respectively.

Detailed Description of the Preferred Embodiment

A. Definitions

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. See, *e.g.* Singleton *et al., Dictionary of Microbiology and Molecular Biology 2nd ed.*, J. Wiley & Sons (New York, NY 1994); Sambrook *et al., Molecular Cloning, A Laboratory Manual,* Cold Springs Harbor Press (Cold Springs Harbor, NY 1989). For purposes of the present invention, the following terms are defined below.

As used herein, the term "CA IX" and "AX014" when used herein represent the tumor-associated antigen that is also an enzyme belonging to the carbonic anhydrase (CA) family. CA IX is also referred to in the art as "MN". For purposes of this invention, from here on, "CA IX" refers to both CA IX and MN. More specifically, CA IX is a transmembrane glycoprotein with an active extracellular enzyme site. Further "AX014H" when used herein refers to human AX014.

"Polymerase chain reaction" or "PCR" refers to a procedure or technique in which minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. patent No. 4,683,195 issued July 28, 1987. Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers can coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and cDNA transcribed from total cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis *et al., Cold Spring Harbor Symp. Quant. Biol.* 51:263 (1987); Erlich, ed., *PCR Technology* (Stockton Pres, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample comprising the use of a known nucleic acid as a primer and a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid.

"Tumor", as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (*e.g.* epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial cancer or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

"Native antibodies and immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains (Chothia *et al. J. Mol. Biol.* 186:651 (1985; Novotny and Haber, *Proc. Natl. Acad. Sci. U.S.A.* 82:4592 (1985); Chothia *et al., Nature* 342:877-883 (1989)).

The term "antibody" herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments, so long as they exhibit the desired biological activity.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called κ and λ, based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes". There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "antibody" includes all classes and subclasses of intact immunoglobulins. The term "antibody" also covers antibody fragments. The term "antibody" specifically covers monoclonal antibodies, including antibody fragment clones.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al, Nature,* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.*, 222:581-597 (1991), for example.

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

By "neutralizing antibody" is meant an antibody molecule which is able to eliminate or significantly reduce an effector function of a target antigen to which is binds. Accordingly, a "neutralizing" anti-CA IX antibody is capable of eliminating or significantly reducing an effector function, such as CA IX enzyme activity.

Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which non-specific cytotoxic cells that express Fc receptors (FcRs) (*e.g.* Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362, or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo, e.g.*, in a animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1988).

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.* (1991). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

Fv" is the minimum antibody fragment which contains a complete antigen-recognition and binding site. In a two-chain Fv species, this region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer

antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.* residues 24-34 (L1), 50-62 (L2), and 89-97 (L3) in the light chain variable domain and 31-55 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al., Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*e.g.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 ((H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol* 196:901-917 (1987)). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

The term "complementarity determining regions" or "CDRs" when used herein refers to parts of immunological receptors that make contact with a specific ligand and determine its specificity. The CDRs of immunological receptors are the most variable part of the receptor protein, giving receptors their diversity, and are carried on six loops at the distal end of the receptor's variable domains, three loops coming from each of the two variable domains of the receptor.

The term "epitope" is used to refer to binding sites for (monoclonal or polyclonal) antibodies on protein antigens.

The term amino acid or amino acid residue, as used herein, refers to naturally occurring L amino acids or to D amino acids as described further below with respect to variants. The commonly used on- and three-letter abbreviations for amino acids are used herein (Bruce Alberts *et al., Molecular Biology of the Cell,* Garland Publishing, Inc., New York (3d ed. 1994)).

The term "disease state" refers to a physiological state of a cell or of a whole mammal in which an interruption, cessation, or disorder of cellular or body functions, systems, or organs has occurred.

The term "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures ,wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of

treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

A "disorder" is any condition that would benefit from treatment of the present invention. This includes chronic and acute disorders or disease including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors, leukemias and lymphoid malignancies, in particular breast, rectal, ovarian, renal, pancreatic, stomach, endometrial, salivary gland, kidney, colon, thyroid, pancreatic, prostate or bladder cancer. A preferred disorder to be treated in accordance with the present invention is malignant tumor, such as cervical carcinomas and cervical intraepithelial squamous and glandular neoplasia, renal cell carcinoma (RCC), esophageal tumors, and carcinomas of epithelial origin.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

"Lipofection" refers to a practical nonviral method for introduction of genetic information into target tissues. Nonviral methods include both chemical or physical methods. Lipofection uses an electrostatically bonded complex of positively charged lipids and negatively charged DNA as a vector which fuses with the cell membrane and delivers DNA into the cytoplasm. Lipofection differs from viral methods in that the efficiency of transfer of genetic information by lipofection is lower than by viral vectors and that the expression of the gene is transient. Alternatively, the complex of lipid and DNA is more stable and easier to handle when compared to viral vectors.

A "plaque assay" specifically refers to the ability of a single, antibody-producing plasma cell to initiate killing of target cells. For example, B cells secrete IgM antibody to antigenic determinants present on the surface of target erythrocytes with T-cell help, resulting in antibody-erythrocyte binding. The presence of an adequate complement source allows adequate complement-mediated lysis of the antibody-coated erythrocytes, resulting in the formation of clear-zones or "plaques" in the agar. Located within the center of each plaque is a single, antibody-producing plasma cell.

B. Methods for carrying out the invention

Embodiments of the invention relate to antibodies directed against the CA IX tumor antigen and methods and means for making and using such antibodies. The present invention provides antibodies that affect the ability of the tumor cell marker, CA IX/MN, to function in tumor cell progression.

In one embodiment, antibodies against CA IX are specifically directed to the catalytic domain of CA IX. Since a number of antibodies specific for other regions of CA IX, such as the adhesion domain (Zavada et al., *Br. J. Cancer*, 82(11):1808-13 (2000))., and with distinct affects on CA IX, methods for distinguishing such antibodies from those that particularly bind the catalytic

domain of CA IX are herein described. In one aspect, the present invention includes methods for screening for such antibodies specifically directed to the catalytic domain of CA IX. For example, experiments detailed below in Example 2, may be of use for screening such particular antibodies directed to the catalytic domain of CA IX. Specifically, assays for measuring the ability of antibodies to block the catalytic function of CA IX or in some way inhibit CA IX to perform its catalytic function, for example, by promoting internalization of CA IX into cells so that CA IX is no longer able to perform its catalytic function on the surface of cells such as tumor cells are included. Such assays may be of use for distinguishing antibodies that bind the catalytic domain and affect the catalytic activity of CA IX from antibodies that function in a different manner. In a further aspect, screening for binding of antibodies that bind to the catalytic domain of CA IX may be performed using the catalytic domain (CD) of CA IX as a target in ELISA screening, further described in Example 2.

In another particular embodiment, the antibodies of the present invention are directed for use in human therapy against diseases. Disease include diseases associated with abnormal cell growth such as tumors. More specifically, diseases may include diseases that rely on the catalytic activity of CA IX for survival and progression. In a particular aspect of the invention, antibodies that are able to inhibit the role of CA IX/MN in cell growth and progression, more specifically tumor cell growth, survival and progression, find use in the treatment of such diseases characterized by abnormal cell growth, in particular, tumors, carcinomas such as renal cell carcinoma and cervical carcinoma, and cancers.

1. Generation of anti-CA IX antibodies

A description follows as to exemplary techniques for the production of the antibodies used in accordance with the present invention.

(a) Monoclonal antibodies

Monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al., Nature* 256: 495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or macaque monkey, is immunized as herein above described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes or more preferably, lymphocytes enriched for B cells then are fused with myeloma cells by an electrocell fusion process or by using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103, [Academic Press, 1996]).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine

guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOP-21 and MC.-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.* 133: 3001 (1984); Brodeur *et al., Monoclonal Antibody Production Techniques and Applications*, pp. 51-63, Marcel Dekker, Inc., New York, [1987]).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al., Anal. Biochem.* 107: 220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the cells may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103, Academic Press, 1996). Suitable culture media for this purpose include, for example, DMEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that

manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of an anti-CA IX monoclonal antibody herein.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody of the invention, or they are substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an CA IX and another antigen-combining site having specificity for a different antigen.

Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

(b) Human antibodies

Attempts to use the same technology for generating human mAbs have been hampered by the lack of a suitable human myeloma cell line. The best results were obtained using heteromyelomas (mouse x human hybrid myelomas) as fusion partners (Kozbor, *J. Immunol.* 133: 3001 (1984); Brodeur, *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp.51-63, Marcel Dekker, Inc., New York, 1987). Alternatively, human antibody-secreting cells can be immortalized by infection with the Epstein-Barr virus (EBV). However, EBV-infected cells are difficult to clone and usually produce only relatively low yields of immunoglobulin (James and Bell, *J. Immunol. Methods* 100: 5-40 [1987]). In future, the immortalization of human B cells might possibly be achieved by introducing a defined combination of transforming genes. Such a possibility is highlighted by a recent demonstration that the expression of the telomerase catalytic subunit together with the SV40 large T oncoprotein and an oncogenic allele of H-*ras* resulted in the tumorigenic conversion of normal human epithelial and fibroblast cells (Hahn *et al.*, *Nature* 400: 464-468 [1999]).

It is now possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production (Jakobovits *et al.*, *Nature* 362: 255-258 [1993]; Lonberg and Huszar, *Int. Rev. Immunol.* 13: 65-93 [1995]; Fishwild *et al.*, *Nat. Biotechnol.* 14: 845-851 [1996]; Mendez *et al.*, *Nat. Genet.* 15: 146-156 [1997]; Green, *J. Immunol. Methods* 231: 11-23 [1999]; Tomizuka *et a l.*, *Proc. Natl. Acad. Sci. USA* 97: 722-727 [2000]; reviewed in Little *et al.*, *Immunol. Today* 21: 364-370 [2000]). For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production (Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551-2555 [1993]). Transfer of the human germ-line immunoglobulin gene array in such germ-line

mutant mice results in the production of human antibodies upon antigen challenge (Jakobovits *et al.*, *Nature* 362: 255-258 [1993]).

Mendez *et al.* (*Nature Genetics* 15: 146-156 [1997]) have generated a line of transgenic mice designated as "XenoMouse® II" that, when challenged with an antigen, generates high affinity fully human antibodies. This was achieved by germ-line integration of megabase human heavy chain and light chain loci into mice with deletion into endogenous J_H segment as described above. The XenoMouse® II harbors 1,020 kb of human heavy chain locus containing approximately 66 V_H genes, complete D_H and J_H regions and three different constant regions (μ, δ and γ), and also harbors 800 kb of human κ locus containing 32 Vκ genes, Jκ segments and Cκ genes. The antibodies produced in these mice closely resemble that seen in humans in all respects, including gene rearrangement, assembly, and repertoire. The human antibodies are preferentially expressed over endogenous antibodies due to deletion in endogenous J_H segment that prevents gene rearrangement in the murine locus.

Such a XenoMouse may be immunized with an antigen of particular interest. Sera from such immunized animals may be screened for antibody-reactivity against the initial antigen. Lymphocytes may be isolated from lymph nodes or spleen cells and may further be selected for B cells by selecting for CD138-negative and CD19+ cells. In one aspect, such B cell cultures (BCCs) may be fused to myeloma cells to generate hybridomas as detailed above. In another aspect, such B cell cultures may be screened further for reactivity against the initial antigen, preferably CA IX protein. Such screening includes ELISA with CA IX-His protein, a competition assay with known antibodies that bind the antigen of interest, such as antibody G250, and in vitro binding to transiently transfected CHO or other cells that express full length CA IX. Such screens are further described in the Examples. To isolate single B cells secreting antibodies of interest, a CA IX-specific hemolytic plaque assay is performed. Cells targeted for lysis are preferably sheep red blood cells (SRBCs) coated with the CA IX antigen. In the presence of a B cell culture secreting the immunoglobulin of interest and complement, the formation of a plaque indicates specific CA IX-mediated lysis of the target cells. The single antigen-specific plasma cell in the center of the plaque can be isolated and used for isolation of mRNA. Using reverse-transcriptase PCR, the DNA encoding the variable region of the antibody secreted can be cloned. Such cloned DNA can then be further inserted into a suitable expression vector, preferably a vector cassette such as a pcDNA, more preferably such a pcDNA vector containing the constant domains of immunglobulin heavy and light chain. The generated vector can then be transfected into host cells, preferably CHO cells, and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the

ordinarily skilled artisan, for example, $CaPO_4$ precipitation and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

In one particular embodiment, the present invention includes a human anti-CA IX monoclonal antibody heavy chain or a fragment thereof, comprising at least one of the following CDR's (as defined by Kabat *et al., Sequences of Proteins of Immunological Interest,* Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3): (a) CDR1 having the sequence of amino acids 26 to 35 of SEQ ID NOs: 2-6, 24-29 125, 130, 133, 142, 150, amino acids 20 to 29 of SEQ ID NO: 30, amino acids 26 to 37 of SEQ ID NOs: 8-19, 22, 128, 136, 137, 146 or 153, amino acids 24 to 35 of SEQ ID NO: 21, amino acids 31 to 35 of SEQ ID NOs: 71-73, 77 or 79 or amino acids 31 to 37 of SEQ ID NO: 75; (b) CDR2 having the sequence of amino acids 50 to 65 of SEQ ID NOs: 2-6, amino acids 52 to 67 of SEQ ID NOs: 8-19 or 22, amino acids 50 to 65 of SEQ ID NOs: 21, amino acids 50 to 65 of SEQ ID NOs: 24-29, amino acids 44 to 59 of SEQ ID NO: 30, amino acids 50 to 65 of SEQ ID NOs: 71-73, 77 or 79, amino acids 52 to 67 of SEQ ID NO: 75, 128, 146 or 153, amino acids 52 to 69 of SEQ ID NO: 136, amino acids 52 to 69 of SEQ ID NO: 137 or amino acids 50 to 66 of SEQ ID NO: 125, 130, 133, 142 or 150, and/or (c) CDR3 having the sequence of amino acids 98 to 109 of SEQ ID NO: 2, 4 or 5, amino acids 98 to 111 of SEQ ID NOs: 3 or 6, amino acids 100-114 of SEQ ID NOs: 8, 11 or 16, amino acids 100-112 of SEQ ID NOs: 9, 10, 12, 17, 18 or 19 amino acids 100-109 of SEQ ID NO: 13, amino acids 100-116 of SEQ ID NO: 14, amino acids 100-111 of SEQ ID NO: 15, amino acids 98 to 104 of SEQ ID NO: 21, amino acids 100-106 of SEQ ID NO: 22, amino acids 98 to 111 of SEQ ID NOs: 24, 25, 26, 29, amino acids 98 to 113 of SEQ ID NO: 27, amino acids 98 to 112 of SEQ ID NO: 28, amino acids 92 to 107 of SEQ ID NO: 30, amino acids 98 to 111 of SEQ ID NOs: 71-73, amino acids 100 to 113 of SEQ ID NO: 75, amino acids 98 to 105 of SEQ ID NO: 77, amino acids 98 to 107 of SEQ ID NO: 79, amino acids 102 to 109 of SEQ ID NO: 136 or 137, amino acids 100 to 109 of SEQ ID NO: 128 amino acids 100 to 114 of SEQ ID NO: 153, amino acids 99 to 109 of SEQ ID NO: 130, amino acids 99 to 116 of SEQ ID NO: 150, amino acids 99 to 115 of SEQ ID NO: 133, amino acids 99 to 113 of SEQ ID NO: 125, amino acids 99 to 106 of SEQ ID NO: 142 or amino acids 100 to 111 of SEQ ID NO: 146. The scope of the invention also covers the heavy chain variable domain of such anti-CA IX monoclonal antibodies. Such heavy chain variable domain can include the entire sequence of SEQ ID NOs: 2-6, 8-10, 21-22, 24-30, 71-73, 75, 77, 79, 136, 128, 153, 137, 130, 150, 133, 125, 142 or 146.

In yet another aspect, the invention provides an anti-human CA IX monoclonal antibody light chain or a fragment thereof, comprising the following CDR's: (a) CDR1 having the sequence of amino acids 23 to 39 of SEQ ID NOs: 32-34, amino acids 24 to 35 of SEQ ID NOs: 36-46, amino acids 24 to 34 of SEQ ID NOs: 48-58, 60-64, 66-69; amino acids 24 to 35 of SEQ ID NOs: 81-83 or amino acids 24 to 34 of SEQ ID NOs: 85, 87, or 89 or amino acids 24 to 39 of SEQ ID

NO: 162 or 168 (b) CDR2 having the sequence of amino acids 55 to 61 of SEQ ID NOs: 32-34, amino acids 51 to 57 of SEQ ID NOs: 36-46, amino acids 50 to 56 of SEQ ID NOs: 48-58, 60-64 or 66-69; amino acids 51 to 57 of SEQ ID NOs: 81-83,amino acids 50 to 56 of SEQ ID NOs: 85, 87 or 89 or amino acids 55 to 61 of SEQ ID NO: 162 or 168, and/or (c) CDR3 having the sequence of amino acids 94 to 102 of SEQ ID NOs: 32-34, amino acids 90 to 97 of SEQ ID NOs: 36, 38, 40, 44, 45, amino acids 90 to 98 of SEQ ID NOs: 37, 39, 42, 43, amino acids 90 to 99 of SEQ ID NO: 41 or 46, amino acids 88 to 96 of SEQ ID NOs: 48-58, amino acids 89 to 97 of SEQ ID NOs: 60-64 or 66-69, amino acids 90 to 97 of SEQ ID NOs: 81-83, amino acids 89 to 96 of SEQ ID NOs: 85, 87 or 89, amino acids 94 to 103 of SEQ ID NO: 162 or amino acids 94 to 102 of SEQ ID NO: 168. The scope of the invention also covers the light chain variable domain of such anti-CA IX monoclonal antibodies. Such light chain variable domain can include the entire sequence of SEQ ID NOs: 32-34, 36-66, 48-58, 60-64, 66-69, 81-83, 85, 87, 89, 162 or 168.

In a further aspect, the invention provides an anti-human CA IX monoclonal antibody comprising (A) at least one heavy chain or a fragment thereof, comprising the following CDR's: (a) CDR1 having the sequence of amino acids 26 to 35 of SEQ ID NOs: 2-6, 24-29 125, 130, 133, 142, 150, amino acids 20 to 29 of SEQ ID NO: 30, amino acids 26 to 37 of SEQ ID NOs: 8-19, 22, 128, 136, 137, 146 or 153, amino acids 24 to 35 of SEQ ID NO: 21, amino acids 31 to 35 of SEQ ID NOs: 71-73, 77 or 79 or amino acids 31 to 37 of SEQ ID NO: 75; (b) CDR2 having the sequence of amino acids 50 to 65 of SEQ ID NOs: 2-6, amino acids 52 to 67 of SEQ ID NOs: 8-19 or 22, amino acids 50 to 65 of SEQ ID NOs: 21, amino acids 50 to 65 of SEQ ID NOs: 24-29, amino acids 44 to 59 of SEQ ID NO: 30, amino acids 50 to 65 of SEQ ID NOs: 71-73, 77 or 79, amino acids 52 to 67 of SEQ ID NO: 75, 128, 146 or 153, amino acids 52 to 69 of SEQ ID NO: 136, amino acids 52 to 69 of SEQ ID NO: 137 or amino acids 50 to 66 of SEQ ID NO: 125, 130, 133, 142 or 150, and/or (c) CDR3 having the sequence of amino acids 98 to 109 of SEQ ID NO: 2, 4 or 5, amino acids 98 to 111 of SEQ ID NOs: 3 or 6, amino acids 100-114 of SEQ ID NOs: 8, 11 or 16, amino acids 100-112 of SEQ ID NOs: 9, 10, 12, 17, 18 or 19 amino acids 100-109 of SEQ ID NO: 13, amino acids 100-116 of SEQ ID NO: 14, amino acids 100-111 of SEQ ID NO: 15, amino acids 98 to 104 of SEQ ID NO: 21, amino acids 100-106 of SEQ ID NO: 22, amino acids 98 to 111 of SEQ ID NOs: 24, 25, 26, 29, amino acids 98 to 113 of SEQ ID NO: 27, amino acids 98 to 112 of SEQ ID NO: 28, amino acids 92 to 107 of SEQ ID NO: 30, amino acids 98 to 111 of SEQ ID NOs: 71-73, amino acids 100 to 113 of SEQ ID NO: 75, amino acids 98 to 105 of SEQ ID NO: 77, amino acids 98 to 107 of SEQ ID NO: 79, amino acids 102 to 109 of SEQ ID NO: 136 or 137, amino acids 100 to 109 of SEQ ID NO: 128 amino acids 100 to 114 of SEQ ID NO: 153, amino acids 99 to 109 of SEQ ID NO: 130, amino acids 99 to 116 of SEQ ID NO: 150, amino acids 99 to 115 of SEQ ID NO: 133, amino acids 99 to 113 of SEQ ID NO: 125, amino acids 99 to 106 of SEQ ID NO: 142 or amino acids 100 to 111 of SEQ ID NO: 146, and/or (B) at least one light chain or a fragment thereof, comprising the following CDR's: (a) CDR1 having the sequence of amino acids 23 to 39 of

SEQ ID NOs: 32-34, amino acids 24 to 35 of SEQ ID NOs: 36-46, amino acids 24 to 34 of SEQ ID NOs: 48-58, 60-64, 66-69; amino acids 24 to 35 of SEQ ID NOs: 81-83 or amino acids 24 to 34 of SEQ ID NOs: 85, 87, or 89 or amino acids 24 to 39 of SEQ ID NO: 162 or 168 (b) CDR2 having the sequence of amino acids 55 to 61 of SEQ ID NOs: 32-34, amino acids 51 to 57 of SEQ ID NOs: 36-46, amino acids 50 to 56 of SEQ ID NOs: 48-58, 60-64 or 66-69; amino acids 51 to 57 of SEQ ID NOs: 81-83,amino acids 50 to 56 of SEQ ID NOs: 85, 87 or 89 or amino acids 55 to 61 of SEQ ID NO: 162 or 168, and/or (c) CDR3 having the sequence of amino acids 94 to 102 of SEQ ID NOs: 32-34, amino acids 90 to 97 of SEQ ID NOs: 36, 38, 40, 44, 45, amino acids 90 to 98 of SEQ ID NOs: 37, 39, 42, 43, amino acids 90 to 99 of SEQ ID NO: 41 or 46, amino acids 88 to 96 of SEQ ID NOs: 48-58, amino acids 89 to 97 of SEQ ID NOs: 60-64 or 66-69, amino acids 90 to 97 of SEQ ID NOs: 81-83, amino acids 89 to 96 of SEQ ID NOs: 85, 87 or 89, amino acids 94 to 103 of SEQ ID NO: 162 or amino acids 94 to 102 of SEQ ID NO: 168. In one aspect, the present invention includes antibodies generated such as AB-MN-21.5.2, AB-MN-21.6.1, AB-MN-22.7, AB-MN-22.8.1, AB-MN-21.17.1, AB-MN-21.8.1, AB-MN-22.11, AB-MN-22.19, AB-MN-22.26, AB-MN-22.27, AB-MN-22.3, AB-MN-22.4, AB-MN-22.5, AB-MN-22.9, AB-MN-21.2.1, AB-MN-21.7.1, AB-MN-22.10, AB-MN-22.14, AB-MN-22.18, AB-MN-22.20, AB-MN-22.21, AB-MN-22.24, AB-MN-22.25, AB-MN-22.28.1, AB-MN-22.30, AB-MN-21.1.1, AB-MN-21.14.1, AB-MN-22.12, AB-MN-22.17, AB-MN-22.29, AB-MN-21.9.1, AB-MN-22.15, AB-MN-22.16, AB-MN-22.23, AB-MN-22.17.1, AB-MN-21.10.1, AB-MN-22.10, AB-MN-22.14, AB-MN-22.20, AB-MN-22.24, AB-MN-22.28.1, AB-MN-22.30, AB-MN-22.7, AB-MN-21.8.1, AB-MN-22.4 and AB-MN-22.13.1 wherein the "AB-MN-21." and "AB-MN-22." designations represent the fact that the antibody was isolated from IgG1 Xenomice or IgG2 Xenomice, respectively, that were immunized with CA IX antigen. The B cells were isolated from the immunized Xenomice and cultured in vitro or fused with a myeloma cell. As described above, the supernatants from the B cell cultures were analyzed for CA IX antibodies. Using a rosette assay, the one B cell making the desired anti-CA IX antibody was identified and the RNA was isolated. The mRNA encoding for the desired antibody was cloned and expressed in a CHO cell. Alternatively, the antibody containing supernates from the myeloma fusion were screened directly using an ELISA assay. The number following "AB-MN-21." or "AB-MN-22.", for example the "21.5.2" in "AB-MN-21.5.2", correlates with the sequence identifed from a hybridoma cell derived from an IgG1 xenomouse. The designation AB-MN 22_indicates that the sequence was derived from a hybridoma derived from an IgG2 immunized mouse . Further, the number following "AX014H", for example the 21_5_2 in "AX014H21_5_2" also correlates with the sequence identified as single cell "21.5.2." The present invention further includes antibodies, such as AB-MN-XG2-109, AB-MN-XG2-051, AB-MN-XG2-023, AB-MN-XG1-080, AB-MN-XG1-141, AB-MN-XG1-174, AB-MN-XG2-109, AB-MN-XG2-051 and AB-MN-XG2-023. "XG1" and "XG2" antibodies were generated by taking lymphocytes from Xenomice immunized with CA IX. The lymphocytes were fused with myeloma

cells to generate hybridomas expressing the CA IX antibodies as described above. The CDR and FR regions in the variable regions of the AB-MN-XG1-109 and AB-MN-XG2-109 are identical. The CDR and FR regions in the variable regions of the AB-MN-XG1-051 and AB-MN-XG2-051 are identical. The CDR and FR regions in the variable regions of the AB-MN-XG1-023 and AB-MN-XG2-023 are identical.

(1) Antibodies from hybridomas

The amino acid sequence of the heavy chain and light chain variable region, having V, D and partial J regions or AB-MN-21.5.2 are represented by SEQ ID NO: 9 as shown in Figures 3A-3B and SEQ ID NO: 32 as shown in Figure 8, respectively, for AB-MN-21.6.1 are represented by SEQ ID NO: 10 as shown in Figure 3 and SEQ ID NO: 33 as shown in Figure 8, respectively, for AB-MN-22.8.1 are represented by SEQ ID NO: 30 as shown in Figure 6 and SEQ ID NO: 36 as shown in Figure 10, respectively, for AB-MN-21.17.1 are represented by SEQ ID NO: 2 as shown in Figure 1 and SEQ ID NO: 37 as shown in Figure 10, respectively, for AB-MN-22.11 are represented by SEQ ID NO: 13 as shown in Figure 3 and SEQ ID NO: 39 as shown in Figure 10, respectively, for AB-MN-22.19 are represented by SEQ ID NO: 3 as shown in Figure 1 and SEQ ID NO: 40 as shown in Figure 10, respectively, for AB-MN-21.6.1 are represented by SEQ ID NO: 10 as shown in Figure 3 and SEQ ID NO: 33 as shown in Figure 8, respectively, for AB-MN-22.26 are represented by SEQ ID NO: 28 as shown in Figure 6 and SEQ ID NO: 41 as shown in Figure 10, respectively, for AB-MN-22.27 are represented by SEQ ID NO: 29 as shown in Figure 6 and SEQ ID NO: 42 as shown in Figure 10, respectively, for AB-MN-22.3 are represented by SEQ ID NO: 5 as shown in Figure 1 and SEQ ID NO: 43 as shown in Figure 10, respectively, for AB-MN-22.5 are represented by SEQ ID NO: 6 as shown in Figure 1 and SEQ ID NO: 45 as shown in Figure 10, respectively and for AB-MN-22.9 are represented by SEQ ID NO: 19 as shown in Figure 3 and SEQ ID NO: 46 as shown in Figure 10, respectively.

The amino acid sequence of the heavy chain and light chain variable region for AB-MN-21.2.1 are represented by SEQ ID NOs: 8 as shown in Figures 3A-3B and SEQ ID NO: 48 as shown in Figure 12, respectively, for AB-MN-21.7.1 are represented by SEQ ID NOs: 11 as shown in Figures 3A-3B and SEQ ID NO: 49 as shown in Figure 12, respectively, for AB-MN-22.18 are represented by SEQ ID NOs: 16 as shown in Figures 3A-3B and SEQ ID NO: 52 as shown in Figure 12, respectively, for AB-MN-22.21 are represented by SEQ ID NOs: 17 as shown in Figures 3A-3B and SEQ ID NO: 54 as shown in Figure 12, respectively, for AB-MN-22.25 are represented by SEQ ID NOs: 22 as shown in Figure 5 and SEQ ID NO: 56 as shown in Figure 12, respectively.

The amino acid sequence of the heavy chain and light chain variable regions for AB-MN-21.1.1 are represented by SEQ ID NOs: 24 as shown in Figure 6 and SEQ ID NO: 60 as shown in Figure 14, respectively, for AB-MN-21.14.1 are represented by SEQ ID NOs: 25 as shown in Figure 6 and SEQ ID NO: 61 as shown in Figure 14, respectively, for AB-MN-22.12 are represented by SEQ ID NOs: 26 as shown in Figure 6 and SEQ ID NO: 62 as shown in Figure 16,

respectively and for AB-MN-22.17 are represented by SEQ ID NO: 27 as shown in Figure 6 and SEQ ID NO: 63 as shown in Figure 16, respectively.

The amino acid sequence of the heavy chain and light chain variable domain for AB-MN-21.9.1 is represented by SEQ ID NOs: 12 as shown in Figures 3A-3B and SEQ ID NO: 66 as shown in Figure 16, respectively, for AB-MN-22.15 is represented by SEQ ID NOs: 14 as shown in Figures 3A-3B and SEQ ID NO: 67 as shown in Figure 16, respectively, for AB-MN-22.16 is represented by SEQ ID NOs: 15 as shown in Figures 3A-3B and SEQ ID NO: 68 as shown in Figure 16, respectively, and for AB-MN-22.23 is represented by SEQ ID NO: 18 as shown in Figures 3A-3B, AB-MN-22.29 are represented by SEQ ID NO: 4, as shown in Figure 1 and SEQ ID NO: 69 as shown in Figure 14, respectively, for AB-MN-22.17.1 are represented by SEQ ID NO: 102 and SEQ ID NO: 103, respectively, forAB-MN-21.10.1 are represented by SEQ ID NO: 21 as shown in Figure 5 and SEQ ID NO: 162 as shown in Figure 28A-B, respectively and for AB-MN-22.13.1 are represented by SEQ ID NO: 146 as shown in Figure 27A-B and SEQ ID NO: 168 as shown in Figure 29A-B, respectively.

The amino acid sequence of the light chain variable region for AB-MN-22.10 is represented by SEQ ID NO: 50 as shown in Figure 12, for AB-MN-22.14 is represented by SEQ ID NO: 51 as shown in Figure 12, for AB-MN-22.20 is represented by SEQ ID NO: 53 as shown in Figure 12, for AB-MN-22.24 is represented by SEQ ID NO: 55 as shown in Figure 12, for AB-MN-22.28.1 is represented by SEQ ID NO: 57 as shown in Figure 12, for AB-MN-22.30 is represented by SEQ ID NO: 58 as shown in Figure 12, for AB-MN-22.7 is represented by SEQ ID NO: 34 as shown in Figure 8, for AB-MN-21.8.1 is represented by SEQ ID NO: 38 as shown in Figure 10 and for AB-MN-22.4 is represented by SEQ ID NO: 44 as shown in Figure 10.

The amino acid sequence of the heavy chain variable region for AB-MN-22.10, AB-MN-22.14, AB-MN-22.20, AB-MN-22.24, AB-MN-22.28.1, AB-MN-22.30, AB-MN-22.7, AB-MN-21.8.1 and AB-MN-22.4 are represented by the sequences of SEQ ID NO: 136 as shown in Figure 26A-B, SEQ ID NO: 128 as shown in Figure 26A-B, SEQ ID NO: 153 as shown in Figure 27A-B, SEQ ID NO: 137 as shown in Figure 26A-B, SEQ ID NO: 130 as shown in Figure 26A-B, SEQ ID NO: 150 as shown in Figure 27A-B, SEQ ID NO: 133 as shown in Figure 26A-B, SEQ ID NO: 125 as shown in Figure 26A-B, SEQ ID NO: 142 as shown in Figure 26A.

The amino acid sequence of the heavy chain variable domain of the Vgamma/D/J germline sequence of VH4-4, VH4-31, VH4-39 and VH4-59 are represented by SEQ ID NOs: 1as shown in Figure 1, SEQ ID NO: 7 as shown in Figures 3A-3B, SEQ ID NO: 20 as shown in Figure 5 and SEQ ID NO: 23 as shown in Figure 6, respectively. The amino acid sequence of the light chain variable domain of the Vkappa/J germline sequence of VK-A19, VK-A27, VK-A30, VK-L5 and VK-O12 are represented by SEQ ID NO: 31 as shown in Figure 8, SEQ ID NO: 35 as shown in Figure 10, SEQ ID NO: 47 as shown in Figure 12, SEQ ID NO: 59 as shown in Figure 14 and SEQ ID NO: 65 as shown in Figure 16, respectively.

The amino acid sequence and encoding nucleotide sequence of the heavy chain variable domain, which includes the signal peptide and additional carboxy-terminal sequence, for AB-MN-22.13.1 are represented by SEQ ID Nos: 169 and 170, respectively. The amino acid sequence and encoding nucleotide sequence of the heavy chain variable domain, which includes the additional carboxy-terminal sequence, for AB-MN-22.8.1 are represented by SEQ ID Nos: 171 and 172, respectively. The amino acid sequence and encoding nucleotide sequence of the heavy chain variable domain, which includes additional carboxy-terminal sequence, for AB-MN-21.10.1 are represented by SEQ ID Nos: 173 and 174, respectively. The amino acid sequence and encoding nucleotide sequence of the heavy chain variable domain, which includes additional carboxy-terminal sequence, for AB-MN-22.28.1 are represented by SEQ ID Nos: 246 and 245, respectively.

The amino acid sequence and encoding nucleotide sequence of the light chain variable domain, which includes the signal peptide and additional carboxy-terminal sequence, for AB-MN-22.13.1 are represented by SEQ ID Nos: 175 and 176, respectively. The amino acid sequence and encoding nucleotide sequence of the light chain variable domain, which includes the additional carboxy-terminal sequence, for AB-MN-22.8.1 are represented by SEQ ID Nos: 177 and 178, respectively. The amino acid sequence and encoding nucleotide sequence of the light chain variable domain, which includes the signal peptide and additional carboxy-terminal sequence, for AB-MN-21.10.1 are represented by SEQ ID Nos: 179 and 180, respectively. The amino acid sequence and encoding nucleotide sequence of the light chain variable domain, which includes the signal peptide and additional carboxy-terminal sequence, for AB-MN-22.28..1 are represented by SEQ ID NOs: 245 and 237, respectively.

The nucleotide sequence encoding for the heavy chain and light chain variable regions for AB-MN-21.14.1 are represented by SEQ ID NOs: 181 and 213, respectively, for AB-MN-21.17.1 are represented by SEQ ID NO: 182 and 214, respectively, for AB-MN-21.1.1 are represented by SEQ ID NO: 183 and 215, respectively, for AB-MN-21.2.1 are represented by SEQ ID NO: 184 and 216, respectively, for AB-MN-21.5.2 are represented by SEQ ID NO: 185 and 217, respectively, for AB-MN-21.6.1 are represented by SEQ ID NO: 186 and 218, respectively, for AB-MN-21.7.1 are represented by SEQ ID NO: 187 and 219, respectively, for AB-MN-21.8.1 are represented by SEQ ID NO: 188 and 220, respectively, for AB-MN-21.9.1 are represented by SEQ ID NO: 189 and 221, respectively, for AB-MN-22.10 are represented by SEQ ID NO: 190 and 222, respectively, for AB-MN-22.11 are represented by SEQ ID NO: 191 and 223, respectively, for AB-MN-22.12 are represented by SEQ ID NO: 192 and 224, respectively, for AB-MN-22.14 are represented by SEQ ID NO: 193 and 225, respectively, for AB-MN-22.15 are represented by SEQ ID NO: 194 and 226, respectively, for AB-MN-22.16 are represented by SEQ ID NO: 195 and 227, respectively, for AB-MN-22.17 are represented by SEQ ID NO: 196 and 228, respectively, for AB-MN-22.18 are represented by SEQ ID NO: 197 and 229, respectively, for AB-MN-22.19 are represented by SEQ ID NO: 198 and 230, respectively, for AB-MN-22.20 are represented by SEQ

ID NO: 199 and 231, respectively, for AB-MN-22.21 are represented by SEQ ID NO: 200 and 232, respectively, for AB-MN-22.23 are represented by SEQ ID NO: 201 and 233, respectively, for AB-MN-22.24 are represented by SEQ ID NO: 202 and 234, respectively, for AB-MN-22.25 are represented by SEQ ID NO: 203 and 235, respectively, for AB-MN-22.26 are represented by SEQ ID NO: 204 and 236, respectively, for AB-MN-22.27 are represented by SEQ ID NO: 116 and 117, respectively, for AB-MN-22.28.1 are represented by SEQ ID NO: 205 and 237, respectively, for AB-MN-22.29 are represented by SEQ ID NO: 206 and 238, respectively, for AB-MN-22.30 are represented by SEQ ID NO: 207 and 239, respectively, for AB-MN-22.3 are represented by SEQ ID NO: 208 and 240, respectively, for AB-MN-22.4 are represented by SEQ ID NO: 209 and 241, respectively, for AB-MN-22.5 are represented by SEQ ID NO: 210 and 242, respectively, for AB-MN-22.7 are represented by SEQ ID NO: 211 and 243, respectively, for AB-MN-22.9 are represented by SEQ ID NO: 212 and 244, respectively.The nucleotide sequence of the heavy chain and light chain variable region for AB-MN-22.27 are represented by SEQ ID NOs: 116 and 117, respectively and for AB-MN-22.17.1 are represented by SEQ ID NOs: 118 and 119, respectively.

Dendrograms representing the hierarchy of anti-CA IX antibodies with a particular germline rearrangement, such as Vgamma/D/J germline sequence of VH4-4, VH4-31 and VH4-59 are shown in Figures 2, 4, 7, respectively and Vkappa/J germline sequence of VK-A19, VK-A27, VK-A30, VK-L5 and VK-O12 are shown in Figures 9, 11, 13, 15 and 17, respectively.

(2) Antibodies from XenoMax

The amino acid sequence of the heavy chain and light chain variable with the signal sequence for AB-MN-XG1-080 is represented by SEQ ID NOs: 71 and 81, respectively, for AB-MN-XG1-141 is represented by SEQ ID NOs: 72 and 82, respectively, for AB-MN-XG1-174 is represented by SEQ ID NOs: 73 and 83, respectively, for AB-MN-XG1-23 is represented by SEQ ID NOs: 75 and 85, respectively, for AB-MN-XG1-51 is represented by SEQ ID NOs: 77 and 87, respectively, and for AB-MN-XG1-109 is represented by SEQ ID NOs: 79 and 89, respectively. The amino acid sequence of the heavy chain variable domain of the Vgamma/D/J germline sequence of VH4-59/D3-3/JH6b, VH4-30.1/D3-9/JH5b, VH3-21/D5-18/JH4b and VH4-04/D1-20/JH6b are represented as shown in Figure 18 by SEQ ID NOs: 70, 74, 76 and 78, respectively. The amino acid sequence of the light chain variable domain of the Vkappa/J germline sequence of A27/JK4, L19/JK1, A20/JK1 and O2/JK3 are represented as shown in Figure 19 by SEQ ID NOs: 80, 84, 86 and 88.

The amino acid sequence of the entire heavy chain variable domain for AB-MN-XG1-080, AB-MN-XG1-141, AB-MN-XG1-174, AB-MN-XG1-023, AB-MN-XG1-051 and AB-MN-XG1-109 are represented by SEQ ID NOs: 90, 92, 94, 96, 98 and 100, respectively. The nucleotide sequence of the entire heavy chain variable domain for AB-MN-XG1-080, AB-MN-XG1-141, AB-MN-XG1-174, AB-MN-XG1-023, AB-MN-XG1-051 and AB-MN-XG1-109 are represented by SEQ ID NOs: 104, 106, 108, 110, 112 and 114, respectively.

The amino acid sequence of the entire light chain variable domain for AB-MN-XG1-080, AB-MN-XG1-141, AB-MN-XG1-174, AB-MN-XG1-023, AB-MN-XG1-051 and AB-MN-XG1-109 are represented by SEQ ID NOs: 91, 93, 95, 97, 99 and 101, respectively. The nucleotide sequence of the entire light chain variable domain for AB-MN-XG1-080, AB-MN-XG1-141, AB-MN-XG1-174, AB-MN-XG1-023, AB-MN-XG1-051 and AB-MN-XG1-109 are represented by SEQ ID NOs: 105, 107, 109, 111, 113, and 115, respectively.

2. Screening for antibodies with the desired properties

(i) Binding to CA IX Antigen

Thus, for example, the anti-CA IX antibodies of the present invention can be identified by incubating a B cell culture or hybridoma supernatant with purified protein from the extracellular domain of CA IX (amino acids 1-413) that has been bound to a microtiter plate. Specific binding can be detected after washing and adding HRP conjugated goat anti-human Fc antibody and determining the optical density with a plate reader set at OD 492 nm.

In another embodiment B cell culture or hybridoma supernatants can be incubated with CHO cells transiently lipofected with full length MN, or more preferably to the catalytic domain of MN, that have been trypsinized and seeded in dishes. After washing, a secondary labeled antibody specific for anti-human IgG Fc is subsequently incubated to allow visualization of binding by fluorescent microscopy.

In one embodiment, the binding assay is a competitive binding assay, where the ability of a candidate antibody to compete with a known anti-CA IX antibody, such as G250, is evaluated. The assay may be performed in various formats, including the ELISA format also illustrated in the Examples below.

Any suitable competition binding assay known in the art can be used to characterize the ability of a candidate anti-CA IX monoclonal antibody to compete with anti-G250 antibody for binding to CA IX antigen. A routine competition assay is described in *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988). In another embodiment, the CA IX-binding ELISA assay described in Example 2 below could be modified to employ CA IX binding competition between a candidate antibody and another known CA IX binding antibody such as G250. Such an assay could be performed by layering the CA IX on microtiter plates, incubating the layered plates with serial dilutions of unlabeled anti-CA IX antibody or unlabeled control antibody admixed with a select concentration of labeled G250, detecting and measuring the signal from the G250 antibody label, and then comparing the signal measurements exhibited by the various dilution of antibody.

(ii) Inhibition of biological activity of CA IX

An important role of carbonic anhydrases such as CA IX in tumors may include a role in tumor survival or progression. The catalytic activity of CA IX includes the conversion of CO_2 and H_20 to carbonic acid. The presence of CA IX on primarily carcinoma cell lines and malignant

tissues, but not in corresponding normal tissues, suggests a possible role for the enzymatic activity of CA IX in tumor progression, more specifically a role in maintaining the characteristic extracellular acidosis and intracellular alkalosis of tumor cells. This low extracellular pH may serve to protect tumor cells from the infiltration and destructive capabilities of immune cells. Thus inhibition of CA IX by anti-CA IX antibodies may cause tumors to be more susceptible to the normal defense characteristics of the immune system as well as to other standard chemotherapy or antibody based tumor treatments.

In one embodiment, the antibodies of the present invention are subjected to a spectrophotometric assay to assess the ability to inhibit carbonic anydrase activity of CA IX. For example, the substrates of CA IX, CO_2 and H_20, are incubated either in the presence or absence (control) of CA IX with phenol red. The absorption of the solution at 558 nm is determined by a spectrophotometer. The time difference for the complete hydration of CO_2 and conversion of the solution from a red to a yellow color can be determined by a concomitant change in absorption at 558 nm. The difference between the control and control plus CA IX enzyme indicates the activity of carbonic anhydrase. To test the effect of a candidate antibody on CA IX enzymatic activity, the antibody is incubated with the CA IX prior to addition of the CA IX substrates.

In another embodiment, the antibodies of the present invention are tested for the ability to disrupt normal CA IX activity at the site of tumor progression. Normal CA IX activity includes enzymatic activity converting water and carbon dioxide into carbonic acid. The internalization of CA IX normally present on the surface of tumor cells blocks the ability of the enzyme to perform its normal function. Accordingly, the removal of CA IX activity following internalization may cause the tumor to be more susceptible to the immune system as well as to other chemotherapy or antibody based tumor treatments. Preferably, screening of candidate anti-CA IX antibodies for internalization is performed by incubating with cells, such as SKRC10 cells. A labeled secondary antibody is added and internalization is initiated by shifting the cells to 37°C. The level of cell surface and internalized anti-CA IX antibody is determined by fluorescent microscopy.

In another embodiment, the disruption of proliferation of cells is another possible use for anti-CA IX cells in tumor treatment. An antiproliferative affect of anti-CA IX antibodies would be useful for inhibiting the proliferation of tumor or cancer cells which is important for disease progression. To determine the effect of anti-CA IX antibodies of the present invention on cell proliferation of cells,such as HeLa or MDA468, expressing endogenous CA IX, are assayed for integrity of mitochondrial function. Metabolically active cells with proper mitochondrial function are able to reduce the substrate MTS tetrazolium compound (ProMega) into a colored product that is soluble in tissue culture media and has a characteristic absorbance at 490 nm. The conversion is presumably accomplished by NADPH or NADH produced by dehydrogenase enzymes in metabolically active cells. A decrease in mitochondrial function of such CA IX-expressing cells may lead to a decrease in cell viability under stressful conditions.

(iii) Mediation of ADCC

Antibody-dependent cell-mediated cytotoxicity (ADCC) is the killing of antibody-coated target cells by cells with Fc receptors that recognize the Fc region of the bound antibody. Most ADCC is mediated by natural killer (NK) cells that have the Fc receptor FcγRIII or CD16 on their surface. The ability of the present anti-CA IX antibodies to mediate ADCC may be of use as tumor treatments. In one embodiment, target cells, such as HT-29 or SK-RC-52 cells, a human renal cell carcinoma cell line, are labeled with a fluorescent Europium (EuDTPA). Candidate antibodies were diluted and aliquotted into microtiter plates. Target cells are preferably incubated with the antibodies for a period prior to the addition of effector peripheral blood mononuclear cells (PMBC). The ability of anti-CA IX cells to induce lysis of such target cells by inducing the response of PBMCs is measured by the release of EuDTPA into the supernatant using a Multilabel counter.

3. Therapeutic compositions and administration of anti-CA IX antibodies

Therapeutic formulations of the anti-CA IX antibodies of the invention are prepared for storage by mixing antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (*Remington: The Science and Practice of Pharmacy*, 19th Edition, Alfonso, R., ed, Mack Publishing Co. (Easton, PA: 1995)), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

The anti-CA IX antibody to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The anti-CA IX antibody ordinarily will be stored in lyophilized form or in solution.

Therapeutic anti-CA IX antibody compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of anti-CA IX antibody administration is in accord with known methods, e.g. injection or infusion by intravenous, intraperitoneal, intracerebral, subcutaneous, intramuscular, intraocular, intraarterial, intracerebrospinal, or intralesional routes, or by sustained release systems as noted below. Preferably the antibody is given systemically.

Suitable examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al., Biopolymers,* 22: 547-556 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer *et al., J. Biomed. Mater. Res.*, 15: 167-277 (1981) and Langer, *Chem. Tech.,* 12: 98-105 (1982)), ethylene vinyl acetate (Langer *et al., supra*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release anti-CA IX antibody compositions may also include liposomally entrapped antibody. Liposomes containing antibody are prepared by methods known *per se*: DE 3,218,121; Epstein *et al., Proc. Natl. Acad. Sci. USA*, 82: 3688-3692 (1985); Hwang *et al., Proc. Natl. Acad. Sci. USA*, 77: 4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamelar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the optimal antibody therapy.

Anti-CA IX antibody can also be administered by inhalation. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution. Alternatively, anti-CA IX antibody can be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder.

An "effective amount" of anti-CA IX antibody to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, the type of anti-CA IX antibody employed, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the anti-CA IX antibody until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays.

Antibodies specific to tumor antigens such as anti-CA IX are useful in targeting of tumor cells for destruction. For example, ricin, a cellular toxin, is finding unique applications, especially in the fight against tumors and cancer. Implications are being discovered as to the use of ricin in the treatment of tumors. Ricin has been suggested to have a greater affinity for cancerous cells than normal cells (Montfort et al. 1987) and has been often termed as a "magic bullet" for targeting malignant tumors. Toxins such as ricin remain active even if the B chain of the toxin is removed. Accordingly, if the solitary A chain is coupled to a tumor-specific antibody, such as anti-CA IX antibody, the toxin has a specific affinity for cancerous cells over normal cells (Taylorson 1996). For example, ricin immunotoxin has been developed to target the CD5 T-cell antigen often found in T-cell and B-cell malignancies (Kreitman et al. 1998). Other types of toxins such as calichaemycin,

geldanamycin and maytansine have also been show to be effective for treating cancerous cells and these type of molecules could be conjugated to anti-CA- IX antibodies (Proc. Natl. Acad. Sci USA 93: 8618, 1996; J. Ntl Cancer Inst.92: 1573, 2000; Cancer Res. 60: 6089, 2000). Further, the linking of such anti-CA IX antibodies to radioisotopes provides advantages to tumor treatments. Unlike chemotherapy and other forms of cancer treatment, radioimmunotherapy or the administration of a radioisotope-antibody combination directly targets the cancer cells with minimal damage to surrounding normal, healthy tissue. With this "magic bullet," the patient can be treated with much smaller quantities of radioisotopes than other forms of treatment available today.

The patients to be treated with the anti-CA IX antibody of the invention include patients or those with tumors, such as esophageal, pancreatic, colorectal tumors, carcinomas, such as renal cell carcinoma (RCC), cervical carcinomas and cervical intraepithelial squamous and glandular neoplasia, and cancers, such as colorectal cancer, breast cancer, lung cancer, and other malignancies. Patients are candidates for therapy in accord with this invention until such point as no healthy tissue remains to be protected from tumor progression. It is desirable to administer an anti-CA IX antibody as early as possible in the development of the tumor, and to continue treatment for as long as is necessary.

In the treatment and prevention of tumor-associated disorder by an anti-CA IX antibody, the antibody composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the antibody, the particular type of antibody, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of antibody to be administered will be governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat the disorder, including treating chronic autoimmune conditions and immunosuppression maintenance in transplant recipients. Such amount is preferably below the amount that is toxic to the host or renders the host significantly more susceptible to infections.

As a general proposition, the initial pharmaceutically effective amount of the antibody administered parenterally will be in the range of about 0.1 to 50 mg/kg of patient body weight per day, with the typical initial range of antibody used being 0.3 to 20 mg/kg/day, more preferably 0.3 to 15 mg/kg/day. The desired dosage can be delivered by a single bolus administration, by multiple bolus administrations, or by continuous infusion administration of antibody, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve.

As noted above, however, these suggested amounts of antibody are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained, as indicated above. For example, the antibody may be optionally formulated with one or more agents currently used to prevent or treat tumors such as standard- or high-dose

chemotherapy and hematopoietic stem-cell transplantation. The effective amount of such other agents depends on the amount of anti-CA IX antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

Further details of the invention can be found in the following example, which further defines the scope of the invention. All references cited throughout the specification, and the references cited therein.

EXAMPLE 1

Preparation of CA IX Tumor Antigens

In the present study, recombinant CA IX proteins were prepared. The full length CA IX cDNA was cloned by Reverse Transcriptase-PCR (RT-PCR) from human HT-29 cells (ATCC) with primers based on the sequence of Pastorek et al (*Oncogene* 9: 2877-2888 (1994)) NCBI Accession # X66839.

The primers used for the amplification of full length (FL) CA IX are as follows:

Forward primer: 5'-GTACACACCGTGTGCTGGGAC-3' (SEQ ID NO: 120)

Reverse primer: 5'-CCTCAGATGCCTCTGGCTGG-3' (SEQ ID NO: 121)

The ~1.5kb PCR product was cloned into pCR3.1 UNI (Invitrogen). Clone #15 was used as a template for PCR amplification of the extracellular domain (aa1-413 of CA IX with the following primers that incorporate a SalI site in the forward primer and a NheI site in the reverse primer):

Forward primer 5'-ATATTACGTCGACGTACACACCGTGTGCTGGAC-3' (SEQ ID NO: 122)

Reverse primer 5'-CAGCTTAGAGCTAGCCCAGCAGCCAGGCAGGAATTCAGC-3' (SEQ ID NO: 123)

The PCR product was digested with SalI and NheI and ligated into CD147HuIgG2 DHFR expression vector (Abgenix, Fremont, CA) digested with SalI and NheI. Plasmids were transfected into 293 cells using a $CaPO_4$ method. The CA IX ECD-HuIgG2 fusion protein was purified from harvested conditioned media via Protein A chromatography. The full-length protein was also expressed in B300.19 cells to generate B300.19/CA IX.

EXAMPLE 2

ANTI-CA IX ANTIBODIES

A. Antibody Generation

1. Immunization and selection of animals for harvesting by ELISA

Monoclonal antibodies against CA IX were developed by sequentially immunizing XM3B-3 and XMG2 mice with recombinant CA IX antigen. In particular, each mouse was immunized into its' hind footpad with CA IX recombinant antigen, generating a large number of candidate mAbs.

These antibodies were then screened for binding and anti-CA IX activity. The mice were initially boosted, and again boosted 3-4 days later with HT-29 cells expressing CA IX at a concentration of 10^7 cells/mouse. Each mouse was further immunized into each hind footpad six additional times (at 3-4 day intervals) with soluble antigen, specifically 10 μg of CA-IX-IgG2-Fc. Four days after the final boost, sera from the immunized mice were tested by ELISA for titer against purified CA IX antigen bound to microtiter plates.

In some experiments determination of anti-CA IX titer was performed with biotinylated CA IX coated onto plates.. 15-500 μg of CA IX protein was diluted in 1 mL of PBS at a pH of 8.6. 10 μl of 10 mg/mL sulfo-NHS-biotin (Biotin stock in DMSO) was added to the 1 mL CA IX-HIS protein solution for an incubation period of 1 hour at room temperature with rotation. After the 1 hour incubation, the reaction was quenched with 100 μl of saturated Tris and subjected to centrifugation and a minimum of 4 washes to separate free biotin from the biotinylated CA IX-His. The biotinylated CA IX-His (1 μg/mL) was coated onto Sigma Streptavidin plates for 1 hour at room temperature. A control Streptavidin plate was left uncoated for use as a control. The plates were washed five times with distilled water. Sera from the immunized animals were titrated in 2% Milk/PBS at 1:2 dilutions in duplicate from a 1:100 initial dilution. The last well was left blank as a control. The Streptavidin plates were washed five times with distilled water. A goat anti-human IgG Fc-specific HRP-conjugated antibody was added at a final concentration of 1 μg/mL for 1 hour at room temperature. Following five washes with distilled water, the Streptavidin plates were developed with the addition of TMB (?) for 30 minutes and the ELISA was stopped by the addition of 1 M phosphoric acid. The specific titer for CA IX of the sera from the immunized mice was determined from the optical density at 450 nm.

Fremont Mouse I.Ds	Titer 1 sigma SA plate coated at 1ug/ml B-MN-His	Titer 2 Sigma SA plate control (not coated)	Titer 3 B-MnHis on sigma SA plate at 1ug/ml	Titer4 B-MnHis on sigma SA plate at 1ug/ml	Titer 5 Sigma SA plate control (not coated)
L991-1	>1:6400	1:12800	>1:204800	>1:102400	1:1600
L991-2	>1:6400	1:12800	>1:204800	>1:102400	1:1600
L991-3	>1:6400	1:6400	>1:204800	>1:102400	1:1600
L991-4	>1:6400	1:25600	>1:204800	>1:102400	1:3200
L991-5	>1:6400	1:12800	1:102400	>1:102400	1:800
naive	1:6400	1:12800	1:12800	1:3200	1:1600

Table 1: Anti-CA IX titre of CA IX-IgG Fc immunized XenoMouse animals

2. Harvest of lymphocytes from immunized animals

XenoMouse animals (L991-3 and L991-4) were selected for harvest of plasma cells, based on the anti-CA IX titer data described above. A total of 55 plates were initiated using $CD19^+$ B

lymphocytes. These plates were set up from the pooled inguinal, popliteal and para-aortic lymph nodes (50 plates) and splenic B lymphocytes (5 plates). The lymph node cells were cultured at either 500 $CD19^+$ cells/well (30 plates) or 250 $CD19^+$ cells/well (20 plates) and the splenic B lymphocytes were cultured at 500 cells/well. B cell cultures were also set up from uncut peripheral blood mononuclear cells at 2500 cells/well (5 plates).

3. Generation of antibody-producing hybridomas

B cells isolated form pooled inguinal, popliteal and para-aortic lymph nodes and splenic B lymphocytes were used for hybridoma generation. Lymphocytes enriched for B cells were fused by electrocell fusion with P3-X63-Ag8.653 myeloma cells and were subjected to hypoxanthine/azaserine (HA) (Sigma, catalog #A9666) selection. Specifically, the fusion was performed by mixing washed enriched B cells and non-secretory myeloma P3X63Ag8.653 cells purchased from ATCC Catalog #CRL1580 (Kearney et al., *J. Immunol.*, 123:1548-1550 (1979) at a ratio of 1:1. The cell mixture was subjected to centrifugation at 800 g. After complete removal of the supernatant, the cells were treated with 2-4 ml of Pronase solution (CalBiochem, catalog #53702; 0.5 mg/ml in PBS) for less than 2 minutes. 3-5 ml of FBS was added to stop the enzyme activity and the suspension was adjusted to 40 ml total volume using electro cell fusion solution (ECFS) (0.3M Sucrose, Sigma, Catalog #S7903, 0.1mM Magnesium Acetate, Sigma, Catalog #M2545, 0.1mM Calcium Acetate, Sigma, Catalog #C4705). The supernatant was removed after centrifugation and the cells were resuspended in 40 ml ECFS. The cells were washed in ECFS once more and finally resuspended in ECFS to a concentration of $2x10^6$ cells/ml. Electro-cell fusion was performed using a fusion generator, model ECM2001, Genetronic, Inc., San Diego, CA. The fusion chamber size used was 2.0 ml using the following instrument settings:

Alignment condition: voltage: 50 v, time: 50 seconds;

Membrane breaking at: voltage: 3000 v, time: 30 ms; and

Post-fusion holding time: 3 seconds.

The hyridoma culture supernatants were screened by ELISA with a his-V5 tagged version of the CA IX protein. The results from the ELISA screen are presented in Table 6.

4. XenoMax Generation of Antibodies

Alternatively, after immunization and selection of B cells for use in antibody generation, wells with cultured B cells were subjected to a number of screens to determine specificity for CA IX antigen.

(a) ELISA

As mentioned above, cultured B cells were subjected to ELISA with a his-V5 tagged version of the CA IX protein.

Biotinylated CA IX-His was bound to streptavidin plates at 275ng/ml and incubated for 18 hours at 4°C. The plate was washed five times with distilled water, blocked with 250µl of 2%Milk/PBS for 30 minutes at room temperature, washed five times with distilled water, incubated

with 40µl of 2%Milk/PBS and 10µl of B cell supernatant for 1hour at room temperature, washed again five times with distilled water, incubated with 50µl of Gt anti-Human (Fc)-HRP at 1µg/ml for 1 hour at room temperature, washed five times with distilled water, incubated with 50µl of TMB substrate, and to stop the reaction, incubated with 10µL of 1M phosphoric acid in each well. The plate was read at a wavelength of 450 nm.

The first 30 plates (derived from the lymph node B-lymphocytes at 500 cells/well) were screened as described above on CA IX-His protein to identify the antigen-specific wells. The data in Table 2 showed that most of the wells had antigen-specific reactivity (2202/2880 at OD 0.3 or greater - 78%). This indicated that these wells were quite polyclonal and likely would not be useful for subsequent screens. The Lymph Node B lymphocytes seeded at 250 cells/well and the B-lymphocytes derived from peripheral blood and the spleen (Table 3) were also analyzed. These data indicated that these wells were also likely polyclonal (412/1440 at OD 0.3 or greater - 29%).

Table 2: 30 Plates of Lymph Node B Lymphocytes Cultured at 500 cells/well

	Positives above cutoff OD of:													
Tissue	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.5	2.0	2.5	3
Inguinal, Popliteal, Para-Aortic LN (plates 11-30)	945	873	790	719	664	619	576	528	480	437	271	139	44	0
above LN with "new EL485" (plates 1-10)	1844	1589	1412	1265	1132	1031	947	861	768	670	358	148	32	0
Total number positives:	2789	2462	2202	1984	1796	1650	1523	1389	1248	1107	629	287	76	0

Table 3: 10 plates of Lymph Node B-cells cultured at 250 cells/well and 5 plates from spleen and PBMNC

	Positives above cutoff OD of:											
Tissue	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.5	2.0
Pooled Lymph Nodes * @250 cells/well (plates #31-50)	957	405	210	146	108	79	56	40	34	30	11	4
Spleen @500 cells/well (plates #51-55)	475	222	102	33	20	12	11	8	8	7	3	2
PBMNC @2500 cells/well (plates 56-60)	480	235	100	42	16	11	7	6	6	6	2	1
Total Number Positives:	1912	862	412	221	144	102	74	54	48	43	16	7

Rescreening of one plate of the highest OD wells on CA IX-His (Table 4) showed that 67% of the wells repeated at the same OD cutoff. The percentage of positives generated in the initial screen (29%) and the percentage of repeat positives (67%) indicated that our cultures were set up very close to the theoretical limit of polyclonality based on the binomial distribution model (20%).

Table 4: Rescreen of one plate of the highest OD

	Positives above cutoff OD of:											
Tissue	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.5	2.0
All wells	95	75	64	60	57	51	46	41	35	30	14	8

(b) Native binding assay in transiently transfected CHO cells

This assay for determining native binding to CA IX was conducted on CHO cells transiently expressing full length MN. BCC supernatants were qualitatively assessed and ranked

for relative binding by eye. The BCC wells were binned (Table 5) using the combined ELISA, G250 and Native binding assays.

To determine native binding, CHO cells were transiently lipofected with full length MN. After 48 hours, the cells were trypsinized, seeded in Terasaki dishes (5000 cells/well), and cultured overnight at 37 °C. The culture media was removed and cells were stained with 10μL of B-cell culture supernatant for 2 hours on ice. The monoclonal anti-MN antibody 13.15 was included as a positive control; titrating 1:2 from 250ng/mL. An irrelevant XG2 recombinant control was also included in the assay. After the primary antibody incubation, the media was removed and the cells were fixed with 1% Paraformaldehyde (10μL for 20min). The cells were washed twice with 20μL PBS. The secondary antibody (Goat anti-Human IgG Fc Alexa 488 2μg/mL) was incubated on ice with the cells for 1 hour. The cells were washed twice with 20μL PBS. The cells were viewed by fluorescent microscopy.

When antibodies were identified, the plasma cells were identified and isolated using a hemolytic plaque assay and PCR was performed to rescue the heavy and light chain sequences. The sequences were then cloned into standard antibody expression vectors, producing recombinant human antibodies. The antigen binding of the recombinant antibodies was subsequently confirmed.

Table 5: Binning of the B Cell Culture wells based on the ability to bind CA-IX based on ELISA, MN G250 and Native Binding Assays

MN1 PLATE 41	D	1	0.58	0.50	1.39	0.10	2.53	+5	B
MN1 PLATE 47	D	1	0.77	0.91	2.01	0.10	2.75	+5	B
MN1 PLATE 44	D	10	0.78	0.87	1.76	0.08	2.91	+5	B
MN1 PLATE 50	D	2	0.72	0.55	1.14	0.11	0.27	+2	C
MN1 PLATE 48	G	5	0.86	0.72	1.54	0.09	0.30	+1/+2	C
MN1 PLATE 48	E	2	1.28	0.58	1.85	0.09	0.45	+1	C
MN1 PLATE 53	G	8	1.28	2.50	2.15	0.11	0.73		D
MN1 PLATE 45	H	2	0.65	0.63	1.80	0.10	0.77		D
MN1 PLATE 44	H	10	0.73	1.88	2.13	0.09	1.85	+5	
MN1 PLATE 44	H	4	1.05	0.63	1.88	0.09	1.68	+5	
MN1 PLATE 42	C	4	1.14	0.61	2.03	0.11	2.95	+5	
MN1 PLATE 45	C	2	1.62	2.54	2.24	0.09	2.08	+5	

			ELISA screens		MN G250 Competition			Native Binding	
Well			1° Screen OD	2° Screen OD	1° Screen OD	SA control OD	G250 competition OD	Terasaki Results (MN-C+ODs)	Bins
MN1 PLATE 45	C	9	0.87	0.87	2.03	0.10	0.31	+5	A
MN1 PLATE 49	C	8	0.96	1.66	1.60	0.10	0.43	+4	A
MN1 PLATE 41	E	1	0.66	0.55	1.51	0.09	0.44	+3	A
MN1 PLATE 42	E	4	1.26	1.07	1.80	0.10	0.74	+4	A
MN1 PLATE 41	F	6	0.81	1.05	1.65	0.15	1.66	+4	B
MN1 PLATE 49	G	4	0.69	0.99	1.69	0.09	1.83	+4	B
MN1 PLATE 47	H	10	0.73	0.67	1.68	0.10	2.19	+4	B
MN1 PLATE 43	G	10	0.68	0.86	1.94	0.11	2.31	+5	B
MN1 PLATE 50	B	4	1.01	1.08	1.41	0.11	1.52	+2	
MN1 PLATE 48	B	6	1.07	1.72	2.05	0.10	2.17	+2	
MN1 PLATE 41	G	8	1.12	1.33	1.90	0.11	1.12	+2	
MN1 PLATE 41	H	5	1.15	0.70	1.83	0.11	1.84	+2	
MN1 PLATE 41	F	9	1.90	1.75	2.02	0.10	2.15	+2	
MN1 PLATE 43	C	2	0.92	1.02	1.91	0.09	2.60	+1	
MN1 PLATE 50	B	3	1.35	1.76	1.77	0.09	1.76	+1	
MN1 PLATE 45	F	6	3.23	2.20	0.98	0.25	1.72	+1	
MN1 PLATE 46	B	1	1.98	1.30	0.36	0.13	0.50	+0.5	
MN1 PLATE 50	D	5	1.72	1.20	2.04	0.09	2.82	no sample	
MN1 PLATE 47	G	6	2.79	2.92	0.27	0.41	0.26		
MN1 PLATE 49	C	10	0.71	1.73	1.87	0.08	2.57	+3	
MN1 PLATE 44	E	3	0.72	0.97	1.43	0.07	2.28	+3	
MN1 PLATE 47	F	8	0.74	0.68	1.32	0.08	2.35	+3	
MN1 PLATE 47	A	2	0.76	1.24	2.08	0.10	1.90	+3	
MN1 PLATE 41	B	12	0.77	0.81	2.07	0.07	2.60	+3	
MN1 PLATE 43	H	7	1.02	0.97	1.98	0.09	2.50	+3	
MN1 PLATE 46	F	8	1.40	1.20	1.98	0.10	2.63	+3	
MN1 PLATE 46	E	2	2.03	2.06	2.23	0.10	1.72	+2/+3	
MN1 PLATE 43	H	10	0.64	0.58	1.81	0.09	2.38	+2	
MN1 PLATE 47	A	9	0.64	0.73	1.78	0.08	1.22	+2	
MN1 PLATE 44	A	5	0.73	0.83	2.06	0.11	2.62	+2	
MN1 PLATE 46	D	8	0.89	2.02	1.94	0.12	2.35	+2	
MN1 PLATE 44	A	2	1.10	1.49	2.25	0.10	2.53	+4	
MN1 PLATE 44	F	3	1.12	1.27	1.84	0.10	1.74	+4	
MN1 PLATE 42	G	7	1.13	1.35	2.09	0.07	1.20	+4	
MN1 PLATE 42	F	5	1.44	2.10	1.58	0.09	2.00	+4	
MN1 PLATE 45	D	3	1.52	1.34	2.17	0.08	2.71	+4	
MN1 PLATE 46	C	4	1.80	2.30	2.31	0.05	1.29	+4	
MN1 PLATE 47	D	3	0.83	0.56	1.99	0.17	2.79	+3/+4	
MN1 PLATE 46	H	8	0.87	1.25	2.00	0.10	1.94	+3/+4	
MN1 PLATE 48	C	8	0.96	0.73	1.75	0.10	2.34	+3/+4	
MN1 PLATE 49	B	2	0.63	0.79	1.44	0.09	2.34	+3	
MN1 PLATE 47	G	10	0.66	0.89	1.74	0.10	2.13	+3	
MN1 PLATE 44	D	2	1.68	1.45	2.39	0.09	1.95	+5	
MN1 PLATE 44	D	4	2.30	0.95	2.15	0.10	1.94	+5	
MN1 PLATE 45	C	12	0.75	1.40	2.32	0.08	2.51	+4	

Bins

A G250 competers, good native binders
B good native binders, low OD (<0.1)
C G250 competers, poor native binders
D no sample, interesting?

B. Antibody Characterization

Antibodies identified from the XenoMax technology and hybridomas were further characterized by assessing their ability to mediate ADCC, internalize cell surface CA IX and inhibit the enzymatic activity of carbonix anyhydrase. Results from these characterizations are summarized in Table 6.

1. Sequence Alignment

The variable regions of the light chains and heavy chains of the antibodies generated from the XenoMax technology and hybridomas were analyzed for amino acid sequence similarity with germline Vgamma/D/J heavy chain and Vkappa/J light chain sequences. Identity and deletions of the Vgamma and Vkappa sequences, without their signal sequences, with their respective germline sequences are shown by a "-" and a "#", respectively in the figures. Dendrograms representing the similarity with the germlines sequences were also generated.

The sequence alignments of the amino acid sequence of the variable heavy chain regions of anti-CA IX antibodies, AB-MN-21.17.1 (SEQ ID NO: 2), AB-MN-22.19 (SEQ ID NO: 3), AB-MN-22.29 (SEQ ID NO: 4), AB-MN-22.3 (SEQ ID NO: 5), AB-MN-22.5 (SEQ ID NO: 6) with Vgamma/D/J germline sequence of VH4-4 (SEQ ID NO: 1), AB-MN-21.2.1 (SEQ ID NO: 8), AB-MN-21.5.2 (SEQ ID NO: 9), AB-MN-21.6.1 (SEQ ID NO: 10), AB-MN-21.7.1 (SEQ ID NO: 11), AB-MN-21.9.1 (SEQ ID NO: 12), AB-MN-22.11 (SEQ ID NO: 13), AB-MN-22.15 (SEQ ID NO: 14), AB-MN-22.16 (SEQ ID NO: 15), AB-MN-22.18 (SEQ ID NO: 16), AB-MN-22.21 (SEQ ID NO: 17), AB-MN-22.23 (SEQ ID NO: 18), AB-MN-22.9 (SEQ ID NO: 19) with Vgamma/D/J germline sequence of VH4-31 (SEQ ID NO: 7), AB-MN-21.10.1 (SEQ ID NO: 21) and AB-MN-22.25 (SEQ ID NO: 22) with Vgamma/D/J germline sequence of VH4-39 (SEQ ID NO: 20) and AB-MN-21.1.1 (SEQ ID NO: 24), AB-MN-21.14.1 (SEQ ID NO: 25), AB-MN-22.12 (SEQ ID NO: 26), AB-MN-22.17 (SEQ ID NO: 27), AB-MN-22.26 (SEQ ID NO: 28), AB-MN-22.27 (SEQ ID NO: 29), AB-MN-22.8.1 (SEQ ID NO: 30) with Vgamma/D/J germline sequence of VH4-59 (SEQ ID NO: 23) are shown in Figures 1, 3, 5 and 6, respectively. Dendrograms of alignments of the variable heavy chain regions of anti-CA IX antibodies with Vgamma/D/J germline sequence of VH4-4, VH4-31 and VH4-59 are shown in Figures 2, 4 and 7, respectively. Figures 1 and 2 show alignment and dendrogram, respectively, of the variable heavy chain region of anti-CA IX antibodies, AB-MN-21.17.1, AB-MN-22.19, AB-MN-22.29, AB-MN-22.3 and AB-MN-22.5 with VH4-4. Figures 3 and 4 show alignment and dendrogram, respectively, of the variable heavy chain regions of anti-CA IX antibodies, AB-MN-21.2.1, AB-MN-21.5.2, AB-MN-21.6.1, AB-MN-21.7.1, AB-MN-21.9.1, AB-MN-22.11, AB-MN-22.15, AB-MN-22.16, AB-MN-22.18, AB-MN-22.21, AB-MN-22.23 and AB-MN-22.9 with VH4-31. Figure 5 shows alignment of the variable heavy chain regions of anti-CA IX antibodies, AB-MN-21.10.1 and AB-MN-22.25 with VH4-39. Figures 6 and 7 show alignment and dendrogram, respectively, of the variable heavy chain regions of anti-

CA IX antibodies of AB-MN-21.1.1, AB-MN-21.14.1, AB-MN-22.12, AB-MN-22.17, AB-MN-22.26, AB-MN-22.27 and AB-MN-22.8.1 with VH4-59.

The sequence alignments of the amino acid sequence of the variable heavy chain regions of anti-CA IX antibodies, AB-MN-XG1-080 (SEQ ID NO: 71), AB-MN-XG1-141 (SEQ ID NO: 72) and AB-MN-XG1-174 (SEQ ID NO: 73) with germline sequence of Vgamma/D/J germline sequence of VH4-59/D3-3/JH6b (SEQ ID NO: 70), AB-MN-XG1-023 (SEQ ID NO: 75) with germline sequence of Vgamma/D/J germline sequence of VH4-30.1/D3-9/JH5b (SEQ ID NO: 74), AB-MN-XG1-051 (SEQ ID NO: 77) with VH3-21/D5-18/JH4b (SEQ ID NO: 77) and AB-MN-XG1-109 (SEQ ID NO: 79) with VH4-04/D1-20/JH6b (SEQ ID NO: 79) are shown in Figure 18.

The sequence alignments of the amino acid sequence of the variable light chain regions of anti-CA IX antibodies AB-MN-21.5.2 (SEQ ID NO: 32), AB-MN-21.6.1 (SEQ ID NO: 33), AB-MN-22.7 (SEQ ID NO: 34) with the amino acid sequence of Vkappa/J germline sequence of VK-A19 (SEQ ID NO: 31), AB-MN-22.8.1 (SEQ ID NO: 36), AB-MN-21.17.1 (SEQ ID NO: 37), AB-MN-21.8.1 (SEQ ID NO: 38), AB-MN-22.11 (SEQ ID NO: 39), AB-MN-22.19 (SEQ ID NO: 40), AB-MN-22.26 (SEQ ID NO: 41), AB-MN-22.27 (SEQ ID NO: 42), AB-MN-22.3 (SEQ ID NO: 43), AB-MN-22.4 (SEQ ID NO: 44), AB-MN-22.5 (SEQ ID NO: 45), AB-MN-22.9 (SEQ ID NO: 46) with the amino acid sequence of Vkappa/J germline sequence VK-A27 (SEQ ID NO: 35), AB-MN-21.2.1 (SEQ ID NO: 48), AB-MN-21.7.1 (SEQ ID NO: 49), AB-MN-22.10 (SEQ ID NO: 50), AB-MN-22.14 (SEQ ID NO: 51), AB-MN-22.18 (SEQ ID NO: 52), AB-MN-22.20 (SEQ ID NO: 53), AB-MN-22.21 (SEQ ID NO: 54), AB-MN-22.24 (SEQ ID NO: 55), AB-MN-22.25 (SEQ ID NO: 56), AB-MN-22.28.1 (SEQ ID NO: 57), AB-MN-22.30 (SEQ ID NO: 58) with the amino acid sequence of Vkappa/J germline sequence of VK-A30 (SEQ ID NO: 47), AB-MN-21.1.1 (SEQ ID NO: 60), AB-MN-21.14.1 (SEQ ID NO: 61), AB-MN-22.12 (SEQ ID NO: 62), AB-MN-22.17 (SEQ ID NO: 63), AB-MN-22.29 (SEQ ID NO: 64) with the amino acid sequence of Vkappa/J germline sequence of VK-L5 (SEQ ID NON: 59) and AB-MN-21.9.1 (SEQ ID NO: 66), AB-MN-22.15 (SEQ ID NO: 67), AB-MN-22.16 (SEQ ID NO: 68), AB-MN-22.23 (SEQ ID NO: 69) with the amino acid sequence of Vkappa/J germline sequence of VK-O12 (SEQ ID NO: 65) are shown in Figures 8, 10, 12, 14 and 16, respectively. Dendrograms of alignments of the variable light chain regions of anti-CA IX antibodies with anti-CA IX antibodies with Vkappa/J germline sequence of VK-A19, VK-A27, VK-A30, VK-L5 and VK-O12 are shown in Figures 9, 11, 13, 15 and 17, respectively. Figures 8 and 9 show alignment and dendrogram, respectively, of the light chain variable domain of anti-CA IX antibodies of 21.5.2, 21.6.1 and 22.7 with VK-A19. Figures 10 and 11 show alignment and dendrogram, respectively, of the light chain variable domain of anti-CA IX antibodies of AB-MN-22.8.1, AB-MN-21.17.1, AB-MN-21.8.1, AB-MN-22.11, AB-MN-22.19, AB-MN-22.26, AB-MN-22.27, AB-MN-22.3, AB-MN-22.4, AB-MN-22.5 and AB-MN-22.9 with VK-A27. Figures 12 and 13 show alignment and dendrogram, respectively, of the light chain variable domain of anti-CA IX antibodies of AB-MN-21.2.1, AB-MN-21.7.1, AB-MN-22.10, AB-

MN-22.14, AB-MN-22.18, AB-MN-22.20, AB-MN-22.21, AB-MN-22.24, AB-MN-22.25, AB-MN-22.28.1 and AB-MN-22.30 with VK-A30. Figures 14 and 15 show alignment and dendrogram, respectively, of the light chain variable domain of anti-CA IX antibodies of AB-MN-21.1.1, AB-MN-21.14.1, AB-MN-22.12, AB-MN-22.17, and AB-MN-22.29 with VK-L5. Figures 16 and 17 show alignment and dendrogram, respectively, of the light chain variable domain of anti-CA IX antibodies of AB-MN-21.9.1, AB-MN-22.15, AB-MN-22.16 and AB-MN-22.23 with VK-O12.

The sequence alignments of the amino acid sequence of the light chain variable domain of anti-CA IX antibodies, AB-MN-XG1-080 (SEQ ID NO: 81), AB-MN-XG1-141 (SEQ ID NO: 82) and AB-MN-XG1-174 (SEQ ID NO: 83) with Vkappa/J germline sequence of A27/JK4 (SEQ ID NO: 80), AB-MN-XG1-023 (SEQ ID NO: 85) with L19/JK1 (SEQ ID NO: 84), AB-MN-XG1-051 (SEQ ID NO: 88) with A20/JK1 (SEQ ID NO: 87) and AB-MN-XG1-109 (SEQ ID NO: 89) with O2/JK3 (SEQ ID NO: 88) are shown in Figure 19.

Figures 26A-26B show sequence alignments of the amino acid sequences of the variable regions of the heavy chains of CA IX antibodies with the amino acid sequences of the following Vgamma/D/J germline sequences: VHD1-2/D7-27/JH6b (SEQ ID NO: 124), VH4-4/D3-3/JH6b (SEQ ID NO: 126), VH4-61/D3-10/JH4b (SEQ ID NO: 127), VH3-23/D1-26/JH4b (SEQ ID NO: 129), VH4-31/D4-17/JH4b (SEQ ID NO: 131), VH3-30/D3-10/JH4b (SEQ ID NO: 132), VH-4-4/D6-19/JH6b (SEQ ID NO: 134), VH6-1/D1-26/JH4b (SEQ ID NO: 135), VH4-31/D3-10/JH4b (SEQ ID NO: 138), VH4-31/D4-11/JH4b (SEQ ID NO: 139), VH4-31/D2-21/JH6b (SEQ ID NO: 140), VH1-2/D1-26/JH4b (SEQ ID NO: 141), VH4-59/D3-9/JH4b (SEQ ID NO: 143). The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H21_8_1N1G2 (SEQ ID NO: 124) was aligned with VH1-2/D7-27/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_5N1G2 (SEQ ID NO: 6), AX014H22_19N1G2 (SEQ ID NO: 3) and AX014H22_3N1G2 (SEQ ID NO: 5) were aligned with VH4-4/D3-3/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_14N1G2 (SEQ ID NO: 128) was aligned with VH4-61/D3-10/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_28_1N1G2 (SEQ ID NO: 130) was aligned with VH3-23/D1-26/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_23N1G2 (SEQ ID NO: 18) and AX014H22_9N1G2 (SEQ ID NO: 19) were aligned with VH4-31/D4-17/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_7N1G2 (SEQ ID NO: 133) was aligned with VH3-30/D3-10/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H21_17_1N1G2 (SEQ ID NO: 2) and AX014H22_29N1G2 (SEQ ID NO: 4) were aligned with VH4-4/D6-19/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_10N1G2 (SEQ ID NO: 136) and AX014H22_24N1G2 (SEQ ID NO: 137) were aligned with VH6-1/D1-26/JH6b.

The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H21_5_2N1G2 (SEQ ID NO: 9) and AX014H21_6_1N1G2 (SEQ ID NO: 10) were aligned with VH4-31/D3-10/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_21N1G2 (SEQ ID NO: 17) was aligned with VH4-31/D4-11/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_16N1G2 (SEQ ID NO: 15) was aligned with VH4-31/D2-21/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_4N1G2 (SEQ ID NO: 142) was aligned with VH1-2/D1-26/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H21_1_1N1G2 (SEQ ID NO: 24), AX014H22_27N1G2 (SEQ ID NO: 29), AX014H21_14_1N1G2 (SEQ ID NO: 25), AX014H22_17N1G2 (SEQ ID NO: 27), AX014H22_26N1G2 (SEQ ID NO: 28) were aligned with VH4-59/D3-9/JH6b.

Figures 27A-27B show sequence alignments of the amino acid sequences of the variable regions of the heavy chains of CA IX antibodies with the amino acid sequences of the following Vgamma/D/J germline sequences: VH4-31/D5-24/JH2 (SEQ ID NO: 144), VH3-48/JH6b (SEQ ID NO: 145), VH4-31/D3-9/JH6b (SEQ ID NO: 147), VH4-39/JH4b (SEQ ID NO: 148), VH3-33/D3-10/JH6b (SEQ ID NO: 149), VH4-31/D3-10/JH6b (SEQ ID NO: 151), VH4-31/D3-9/JH3b (SEQ ID NO: 152), VH4-59/D6-13/JH6b (SEQ ID NO: 154). The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_11N1G2 (SEQ ID NO: 13) was aligned with VH4-31/D5-24/JH2. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_13_1N1G2 (SEQ ID NO: 146) was aligned with VH3-48/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_15N1G2 (SEQ ID NO: 14) was aligned with VH4-31/D3-9/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_25N1G2 (SEQ ID NO: 22) and AX014H21_10_1N1G2 (SEQ ID NO: 21)_were aligned with VH4-39/JH4b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_30N1G2 (SEQ ID NO: 150) was aligned with VH4-33/D3-10/JH6b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H21_9_1N1G2 (SEQ ID NO: 12) was aligned with VH4-31/D3-10/JH4b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_18N1G2 (SEQ ID NO: 16), AX014H21_7_1N1G2 (SEQ ID NO: 11), AX014H22_20N1G2 (SEQ ID NO: 153) and and AX014H21_2_1N1G2 (SEQ ID NO: 8)_were aligned with VH4-31/D3-9/JH3b. The amino acid sequences of the heavy chain variable domain of CA IX antibodies, AX014H22_8_1N1G2 (SEQ ID NO: 30) and AX014H22_12N1G2 (SEQ ID NO: 26) were aligned with VH4-59/D6-13/JH3b.

Figures 28A-28B show sequence alignments of the amino acid sequences of the variable regions of the light chains of CA IX antibodies with the amino acid sequences of the following Vkappa/J germline sequences: O12/JK4 (SEQ ID NO: 155), A27/JK5 (SEQ ID NO: 156), A3/JK4

(SEQ ID NO: 157), A27/JK4 (SEQ ID NO: 80), A30/JK4 (SEQ ID NO: 158), L5/JK5 (SEQ ID NO: 159), A30/JK3 (SEQ ID NO: 160), A1/JK4 (SEQ ID NO: 161) and A27/JK2 (SEQ ID NO: 163). The amino acid sequences of the light chain variable domain of CA IX antibodies, AX014H22_15N1K (SEQ ID NO: 67), AX014H22_23N1K (SEQ ID NO: 69), AX014H21_9_1N1K (SEQ ID NO: 66), AX014H22_16N1K (SEQ ID NO: 68) were aligned with O12/JK4. The amino acid sequences of the light chain variable domain of CA IX antibodies, AX014H22_3N1K (SEQ ID NO: 43) were aligned with A27/JK5. The amino acid sequences of the light chain variable domain of CA IX antibodies, AX014H21_6_1N1K (SEQ ID NO: 33) and AX014H21_5_2N1K (SEQ ID NO: 32) were aligned with A3/JK4. The amino acid sequences of the light chain variable domain of CA IX antibodies, AX014H22_8_1N1K (SEQ ID NO: 36), AX014H22_27N1K (SEQ ID NO: 42), AX014H22_5N1K (SEQ ID NO: 45), AX014H21_17_1N1K (SEQ ID NO: 37), AX014H22_11N1K (SEQ ID NO: 39), AX014H21_8_1N1K (SEQ ID NO: 38), AX014H22_19N1K (SEQ ID NO: 40), AX014H22_4N1K (SEQ ID NO: 44) were aligned with A27/JK4. The amino acid sequences of the light chain variable domain of CA IX antibodies, AX014H22_30N1K (SEQ ID NO: 58), AX014H22_14N1K (SEQ ID NO: 51 and AX014H22_28_1N1K (SEQ ID NO: 57), were aligned with A27/JK4. The amino acid sequences of the light chain variable domain of CA IX antibodies, AX014H21_1_1N1K (SEQ ID NO: 60), AX014H22_29N1K (SEQ ID NO: 64), AX014H21_14_1N1K (SEQ ID NO: 61), AX014H22_17N1K (SEQ ID NO: 63) were aligned with L5/JK5. The amino acid sequences of the light chain variable domain of CA IX antibodies, AX014H22_24N1K (SEQ ID NO: 55) and AX014H22_10N1K (SEQ ID NO: 50) were aligned with A30/JK3. The amino acid sequence of the light chain variable domain of AX014H21_10_1N1K (SEQ ID NO: 162) was aligned with A1/JK4. The amino acid sequences of the light chain variable domain of CA IX antibodies, AX014H22_9N1K (SEQ ID NO: 46) and AX014H22_26N1K (SEQ ID NO: 41) were aligned with A27/JK2.

Figures 29A-29B show sequence alignments of the amino acid sequences of the variable regions of the light chains of CA IX antibodies with the amino acid sequences of the following Vkappa/J germline sequences: L5/JK3 (SEQ ID NO: 164), A3/JK2 (SEQ ID NO: 165), A30/JK1 (SEQ ID NO: 166) and A23/JK4 (SEQ ID NO: 167). The amino acid sequences of the light chain variable domain of CA IX antibodies, AX014H22_12N1K (SEQ ID NO: 62) was aligned with L5/JK3. The amino acid sequences of the light chain variable domain of CA IX antibodies, AX014H22_7N1K (SEQ ID NO: 34) was aligned with A3/JK2. The amino acid sequences of the light chain variable domain of CA IX antibodies, AX014H22_18N1K (SEQ ID NO: 52), AX014H21_7_1N1K (SEQ ID NO: 49), AX014H22_21N1K (SEQ ID NO: 54), AX014H22_20N1K (SEQ ID NO: 53), AX014H22_25N1K (SEQ ID NO: 56), AX014H21_2_1N1K (SEQ ID NO: 48) were aligned with A30/JK1. The amino acid sequence of

the light chain variable domain of CA IX antibodiey, AX014H22_13_1N1K (SEQ ID NO: 168) was aligned with A23/JK4.

2. HT29 Tumor cell binding assay

Binding of anti-CA IX antibodies to tumor cells was determined by immunofluorescence microscopy HT-29 cells were grown overnight in a Black Walled 96-well dish at 37°C in McCoy's 5a media with 10%FCS. Staining of the cells was performed on ice. AB-hIL8-XG2142 was included as an irrelevant XG2 recombinant control. Mini lipofection supernatants were titrated 1:2 from neet in DMEM with 10% fetal calf serum (FCS). Primary antibody incubation of AB-MN-XG2-109, AB-MN-XG2-051, or AB-MN-XG2-023, was performed on ice for 1 hour, and cells were subsequently washed 2x with 100μL PBS. The cells were incubated with a secondary antibody (Goat anti-Human IgG Fc Alexa) at concentration of 2μg/mL for 1 hour on ice. Cells were further washed twice with 100μL PBS. Cells were fixed with 1% Paraformaldehyde and viewed on Olympus Imaging Station.

Results as shown in Figure 20 show that AB-MN-XG2-109, AB-MN-XG2-023, and AB-MN-XG2-051 were able to bind to HT-29 tumor cells.

3. Proliferation Assay

Anti-CA IX antibodies were tested for their ability to reduce the viability of HeLa cells and MDA 468 cells expressing endogenous CA IX antigen (Figures 21A and 21B). The cell viability assay directly measures mitochondrial function with decreased mitochondrial activity possibly leading to a decrease in cell viability under stressful conditions.

As described in the Promega's manufacturer's protocol, The CellTiter 96 ® AQueous One Solution Cell Proliferation Assay (a) was a colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays. The CellTiter 96 ® AQueous One Solution Reagent contained a novel tetra-zolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sul-fophenyl)-2H-tetrazolium, inner salt; MTS] and an electron coupling reagent (phenazine ethosulfate; PES). PES had enhanced chemical stability, which allowed it to be combined with MTS to form a stable solution. This convenient "One Solution" format was an improvement over the first version of the CellTiter 96 ® AQueous Assay, where phenazine methosulfate (PMS) was used as the electron coupling reagent, and PMS Solution and MTS Solution are supplied separately. The MTS tetrazolium compound (Owen's reagent) was bioreduced by cells into a colored formazan product that is soluble in tissue culture medium. This conversion was presumably accomplished by NADPH or NADH produced by dehydro-genase enzymes in metabolically active cells. Assays were performed by adding a small amount of the CellTiter 96 ® AQueous One Solution Reagent directly to culture wells, incubating for 1–4 hours and then recording absorbance at 490nm with a 96 well plate reader.

As shown in Figure 21A, 21B and 21C, AB-MN-XG1-080, AB-MN-XG1-141, AB-MN-XG1-174, AB-MN-XG1-023, AB-MN-XG1-051 reduced the viability of HeLa cells and MDA 468 cells expressing endogenous CA IX antigen.

4. Internalization Assay

Anti-CA IX antibodies were further tested for their ability to become internalized by SKRC10 cells (Figure 22). Internalization of the antibodies were detected by immunofluorescence microscopy.

SKRC10 cells were seeded at 2-5x10^4 cells/well in an 8-chamber slide for 24-48 hrs. Anti-CA IX primary antibody (5 μg/ml in 2%FBS/PBS) was added to each well and incubated for 1 hour at 4°C (100 μl total volume). The wells were washed three times with cold PBS. Secondary antibody (1 μl goat anti-human AF546, Jackson) was added to the wells for 1 hour at 4°C (100 μl total volume, 2%FBS/PBS). After three washes with cold PBS, internalization was initiated by shifting the chamber slides to 37°C for specific time points (for cell surface staining, cells were left on ice). Internalization was stopped by shifting the cells from 37°C to ice. Cells were fixed in formaldehyde (Cytofix, Pharmingen-should be ~3.7%) for a period of 10 min on ice. Cells were then washed 3 times with cold PBS. DNA was stained with Hoechst (1:10,000 in PBS) for 5 min on ice. The cells were washed three times with cold PBS. Chambers were detached excess buffer was aspirated from the slide. To view cells, 1 drop of mounting medium (Vectashield) was added to each chamber. Cells were viewed under 40X-60X magnification.

As shown in Figure 22, AB-MN-XG1-051 and AB-MN-XG1-023 antibodies were internalized into SKRC10 cells.

5. Antibody-dependent cell-mediated cytotoxicity (ADCC) assay

Peripheral blood mononuclear cells (PBMCs) purified from whole blood over Ficoll-paque were used as effector cells to mediate lysis of antibody sensitized SKRC-52 cells labeled with EuDTPA.

SKRC-52 renal cancer cell lines which express MN antigen were used as target cells and were incubated with various concentrations of anti-CA IX antibodies ranging from 0.0001μg/mL to 10 μg/mL. The cells were cultured in McCoy's 5a medium supplemented with 2mM glutamine, 100 units/mL penicillin, 100μg/mL streptomycin and 10% fetal bovine serum. SKRC-52 cells were washed once with HEPES buffer (containing 50mM Hepes, 93mM NaCl, 5mM KCl and 2mM $MgCl_2$, pH 7.4). 10x10^6 target cells were suspended in 1.0mL of cold labeling buffer containing 600μM $EuCl_3$, 3mM DTPA and 25μg/mL dextran sulphate and incubated on ice for 20 minutes with frequent shaking. Labeling of target cells was terminated with the addition of 9 mL of repair buffer (Hepes buffer containing 2 mM $CaCl_2$ and 10mM D-glucose at pH 7.4) followed by further incubation on ice for 5 minutes. Then the tube was filled up with repairing buffer and the cells are spun down. Labeled cells were washed three times more with repairing buffer and twice with assay

medium (RPMI 1640 medium with 10% fetal bovine serum) and kept on ice until required. Immediately prior to the experiment, the cells were washed once more in assay medium and the cell pellet was transferred to a clean test tube, diluted to 10^5 cells/mL.

Anti-CA IX monoclonal antibodies used in the assay were AB-MN-21.1.1, AB-MN-21.2.1, AB-MN-21.6.1, AB-MN-21.7.1, AB-MN-21.8.1, AB-MN-21.9.1, AB-MN-21.10.1, AB-MN-21.11.1, AB-MN-21.13.1, AB-MN-21.14.1, AB-MN-21.15.1, AB-MN-21.17.1, AB-MN-21.18.1, AB-MN-21.5.2 (IgG1) and AB-MN-22.1, AB-MN-22.22 (IgG2). Antibody controls and samples were diluted in 10 fold dilutions in assay medium and 50 µL volumes pipetted into U–bottomed microtiter plates. The assay blank consisted of medium only. The assay plates containing samples were pre-cooled on ice for 30 minutes. Target cells labeled with EuDTPA as described above were added in 50µL volumes ($0.5x10^4$ cells) and incubated on ice for 30 minutes. Effector cells, peripheral blood mononuclear cells (PBMC) isolated from the interface created upon centrifugation of fresh blood from normal donors on a Ficoll-Paque gradient (Amersham Pharmacia Biotech AB) with a density of 1.0770 g/ml were added in 100µl volumes (E:T ratio of 50:1). The plates were shaken briefly on a plate shaker. ADCC mediated lysis of target cells was induced after pelleting cells by centrifugation at 1800rpm for 5 minutes, followed by incubation at 37^0 C in a humidified CO_2 incubator for 2 hours. The cells were centrifuged again and 20 µL supernatants collected for estimation of EuDTPA release to a flat–bottomed microtiter isoplate, to which was added 200 µL enhancement solution containing 15 µM 2-naphthoyl-trifluoroacetone, 50 µM tri-n-octylphosphine oxide and 0.1% (v/v) Triton X-100. EuDTPA and enhancement solution was mixed for 10 minutes on a shaker. Fluorescence was quantified using a wallac 1420 Multilabel Counter.

The lysis of target cells was determined in the presence of antibody (experimental release), without antibody (spontaneous release) or in 1% Triton X-100 (total release). Percent specific cell lysis was calculated using the equation:

Percent specific lysis =

$$\frac{\text{(experimental release)} - \text{(spontaneous release)}}{\text{(total release)} - \text{(spontaneous release)}} \times 100$$

Figures 23A, 23B and 23C show that as low as 0.01mg/mL anti-CA IX IgG1 antibodies induced a cellular cytotoxicity of about 25-40 % compared to 15-20 % lysis induced by IgG2 antibodies. Anti-CA IX IgG1 antibodies induced a cellular cytotoxicity of about 55-75% with 1µg/mL and 45-60% with 0.1µg/mL antibody concentration compared to 30% and 20% lysis induced by hIgG1 isotype control at 1mg/mL and 0.1mg/mL, respectively using SK-RC-52 target cells.

6. *Carbonic anhydrase spectrophotometric assay*

To determine whether the anti-CA IX antibodies affect the enzymatic activity of CA IX, the antibodies were tested in a spectrophotometric assay. 50 L of carbonic anhydrase or MN protein at 0.01 mg/mL was added to 2.4 ml of phenol red at 12.5 mg/L before mixing with the substrates of CA IX, H_2O and CO_2. As a control, 2.4 ml of phenol red at 12.5 mg/L was mixed with 0.6 mL of water saturated with CO_2 in the absence of carbonic anhydrase or MN protein. Mixing was performed at the T-junction between two syringes, one containing phenol red/carbonic anhydrase and the other water/CO_2. The mixed solution was dispensed into a UV cuvette and the absorbance at 558 nm is recorded. This reaction was monitored at a temperature of about 15°C. Carbonic anhydrase activity was demonstrated by the difference in time for the complete hydration of CO_2 between the control by itself and the control with carbonic anhydrase or MN protein. The completion of the reaction was indicated by the solution changing from red to yellow. To test the effect of an antibody on carbonic anhydrase activity, antibody was incubated with the MN receptor at 2-8°C for 4 hours before it was added to the substrate containing solution. The assay was carried out as described above.

All antibodies that bound CA IX were tested for their ability to inhibit enzyme activity. CA IX antibodies, AB-MN-21.7 and AB-MN-21.17.1, AB-MN-22.8, AB-MN-22.12, AB-MN-22.13, AB-MN-22.27 and AB-MN-22.28 tested positive for the ability to inhibit the carbonic anhydrase activity CA IX (Table 6).

7. *Affinity determination*

Anti-CA-IX antibodies of the present invention were further tested for their affinity for CA IX antigen.

All CA IX antibodies of the present invention were diluted in running buffer with BSA and CM-Dextran (3g/250 ml, respectively) according to their concentration using the following methods: dilution 1/70 with a concentration greater than 0.3 mg/ml; 1/300 dilution with a concentration greater than or equal to 0.3 mg/ml; 1/1500 with a concentration greater than or equal to 0.6 mg/ml; 1/3000 with a concentration greater than or equal to 3.0 mg/ml. Each monoclonal antibody was captured on a separate surface using 5-minute contact, and a 6-minute wash for stabilization of monoclonal antibody. Kinject CA IX antigen at 600 nM over all surfaces for one minute was followed by 2-minute dissociation. Two different antigens were used to determine affinity. One antigen comprised the entire extracellular domain and the other contained only the catalytic domain. At higher concentrations, the protein containing the catalytic domain resulted in cleaner data than the protein containing the entire extracellular domain. However, no DTT was added in the screening process of the affinity of the antibodies for CA IX since the addition of 10 mM DTT abolished binding of both proteins. Binding data was prepared by subtracting the baseline drift of a buffer kinject just prior to the injection of the antigen from the measurements taken after the injection of the antigen. Data were normalized for the amount of CA IX antibody

captured on each surface, and were further fit globally to a 1:1 interaction model to determine binding kinetics. The data are summarized in Table 6.

Table 6: Summary Table of Antibodies that have been Characterized

Antibody Designation	IgG Isotype	CA-IX-His-ECD	CA9-CD Binding	Internalization	ADCC Activity	Inhibits CA9 Enzyme	Affinity ECD/CD
21.1.1 a	**IgG1**	1.643	2.5	Yes	Yes	No	27.4/1.6
21.2.1 b	**IgG1**	1.209	2.485	Yes	Yes	No	36/3.2
21.6.1 c	IgG1	0.618	2.247	Yes	Yes	No	94.9/91
21.7.1 d	IgG1	1.928	2.772	Yes	Yes	Yes	29.9/1.1
21.8.1 e	IgG1	1.61	2.54	Yes	Yes	No	225/24
21.9.1 f	IgG1	1.62	2.533	Yes	Yes	No	417/.09
21.10.1 g	IgG1	0.059	0.187		Yes	Yes	13/NA
21.11.1 h	IgG1	1.195	2.734	Yes	Yes	No	36.9/NA
21.14.1 j	IgG1	1.78	2.473	Yes	Yes	No	18.3/3.0
21.15.1 k	IgG1	1.501	2.629	Yes	Yes	No	
21.17.1 l	**IgG1**	1.853	2.663	Yes	Yes	Yes	NA/3.7
21.18.1 m	IgG1	1.191	2.066	Yes	Yes	No	4.6/35
21.5.2 n	IgG1	0.893	2.427	Yes	Yes	No	120/NA
#141	IgG1	2.562	2.561	Yes		Yes	6/.05
#174	IgG1	2.515	2.505	Yes		No	NA/2.1
#80	**IgG1**	2.705	2.846	Yes		No	16.2/NA
#23	**IgG1**	2.221	2.419	Yes		No	NA/NA
#109	IgG1	1.601	2.898	Yes		No	5.34/3.7
#51	**IgG1**	2.468	2.883	Yes		No	0.27.84
22.7	IgG2	0.097	0.715			No	NA/NA
22.8	**IgG2**	2.406	2.768	Yes		Yes	16.9/NA
22.9	IgG2	0.395	2.276			No	NA/NA
22.10	IgG2	0.057	0.115	Yes		No	NA/NA
22.11	IgG2	1.156	0.79			No	NA/NA
22.12	IgG2	2.381	2.579			Yes	9.78/4.4
22.13	IgG2	1.848	0.118			Yes	18.8/NA
22.14	IgG2	0.763	2.033			No	NA/NA
22.15	IgG2	0.831	2.528			No	8.8/108
22.16	IgG2	0.162	0.525			No	NA/NA
22.17	**IgG2**	2.163	2.568	Yes		No	NA/NA
22.18	IgG2	2.234	2.75	Yes		No	1.1/.05
22.19	**IgG2**	2.123	3.012	Yes		No	NA/NA
22.20	IgG2	1.72	2.707			No	NA/NA
22.21	IgG2	2.176	2.736			No	0.74/0.1
22.22	IgG2	2.445	2.537	Yes		No	NA/NA
22.23	IgG2	2.008	2.692			No	NA/NA
22.24	IgG2	0.085	0.07			No	NA/NA
22.25	IgG2	1.841	2.475			No	NA/NA
22.26	IgG2	1.991	2.747			No	NA/NA
22.27	**IgG2**	1.224	1.84			Yes	162/NA
22.28	**IgG2**	0.404	2.537			Yes	2.75/NA
22.29	IgG2	1.957	2.52			No	NA/NA
22.30	IgG2	2.201	2.464			No	NA/NA
G250						NO	NA/NA

EXAMPLE 3

CA IX EXPRESSION

A. Expression of CA IX in Cancer Cells

To determine whether cancer cells express CA IX, 96 different cancer cells were incubated with anti-CA IX antibody and subsequently incubated with anti-human IgG-HRP in a cell based array (CBA).

The 96 different cancer cells were grown in respective medium, aliquoted into 96 well plates and incubated with 5 μg/ml of CA IX antibody, AB-MN-21.141 for 1 hour. The cells were washed 2 times and then incubated with the secondary antibody, goat anti-human IgG-HRP (Jackson Immunoresearch). After washing the secondary antibody, tetramethylbenzidine (TMB) substrate (Zymed) was added to each well. The OD was measured at 650 nm.

The graphs in Figures 24A and 24B represents the results of CA IX expression in a subset of the cancer cell lines tested in the CBA. Results showing expression levels of human CA IX in melanoma cell lines, SK-mel-5, Hs 936.T, LOX IMVI, M-14 SK-MEL-2, UACC-62 and UACC-257, cervical cancer cell lines, A-431, Hela, HT-3, MS751 and ES2 and and ovarian cancer cell lines, IGROV1, MDAH2774, SK-OV-3, OVCAR-4, OVCAR-5 and OVCAR-8 are shown in Figure 24A. Results showing expression levels of human CA IX in pancreatic cancer cell lines, BxPC3, HPAC, HPAF II and CAPAN-1, , prostate cancer cell lines, DU 145, 22Rv1, LNCaP and PC-3 and renal cancer cell lines, A498, 786-0, SK-RC-01, SK-RC-10, Caki-1, Caki-2, RXF-393, SK-RC-52, SN12C, TK-10, UO-31, MV 522 are shown in Figure 24B.

B. Induced Expression of CA IX In Hypoxic Conditions

To determine whether CA IX expresssion is induced when cells are grown under hypoxic conditions, ovarian (OvCar 3) and cervical (HeLa) cancer cells were cultured under aerobic (A) or hypoxic (H), 0.5% oxygen, conditions for 24 to 48 hours. The cells were harvested and the level of cell surface CA IX expression was determined in a cell based assay (CBA) using 5 μg/ml CA IX antibody, AB-MN-21.141, per well. After incubation with the CA IX antibody for 1 hour, the secondary goat anti-human IgG-HRP was added to the wells. The wells were washed 2 times and further incubated with tetramethylbenzidine (TMB) substate. The OD was measured at 650 nm.

Figure 25 shows induction of CA IX expression in cells cultured in hypoxic conditions.

EXAMPLE 4

Uses of Anti-CA IX Antibodies for Tumor Treatment

Antibodies specific to tumor antigens such as anti-CA IX are useful in targeting of tumor cells expressing such antigens for elimination.

A. Linkage of anti-CA IX antibody to ricin and other toxins

Ricin, a cellular toxin, is finding unique applications, especially in the fight against tumors and cancer. Implications are being discovered as to the use of ricin in the treatment of tumors.

Ricin has been suggested to have a greater affinity for cancerous cells than normal cells (Montfort et al. 1987) and has been often termed as a "magic bullet" for targeting malignant tumors. Toxins such as ricin remain active even if the B chain which is responsible for because of toxin nonspecific lectin activity leads to toxic side effects is removed. Accordingly, if the solitary A chain is coupled to a tumor-specific antibody, the toxin has a specific affinity for cancerous cells over normal cells (Taylorson 1996). For example, ricin immunotoxin has been developed to target the CD5 T-cell antigen often found in T-cell and B-cell malignancies (Kreitman et al. 1998).

A novel method of coupling whole intact ricin to monoclonal antibody is described in Pietersz et al. (*Cancer Res* 48(16):4469-76 (1998)) and includes blocking of nonspecific binding of the ricin B-chain. Coupling of ricin to the anti-CA IX antibodies of the present invention may be done by using the bifunctional reagents S-acetylmercaptosuccinic anhydride for antibody and succinimidyl 3-(2-pyridyldithio)propionate for ricin. The coupling should result in the loss of B-chain binding activity, while impairing neither the toxic potential of the A-chain nor the activity of the antibody. Whole ricin-antibody conjugates produced in this way should not bind nonspecifically to target cells, the most important implication being that such immunotoxins should be more potent that ricin A-chain conjugates and capable of being used in vivo.

Immunoconjugates composed of antibodies coupled to extremely potent drugs have recently been shown to have improved activity against tumor cells. These immunoconjugates are very efficient in delivering toxic drugs such as geldanamycin, calicheamicin and maytansinoids to cells (Proc. Natl. Acad. Sci USA 93: 8618, 1996; J. Ntl Cancer Inst.92: 1573, 2000; Cancer Res. 60: 6089, 2000) and several of these drug- immunoconjugates are in clinical trial. They have the advantage that the small molecule drugs are not immunogenic like ricin and therefore the immunocojugate therapy can be delivered to patients multiple times.

B. Linkage to Radioisotope

The linking of such anti-CA IX antibodies to radioisotopes provides advantages to tumor treatments. Unlike chemotherapy and other forms of cancer treatment, radioimmunotherapy or the administration of a radioisotope-antibody combination directly targets the cancer cells with minimal damage to surrounding normal, healthy tissue. With this "magic bullet," the patient can be treated with much smaller quantities of radioisotopes than other forms of treatment available today. Preferred radioisotopes include yttrium^{90} (90Y), indium^{111} (111In), ^{131}I, ^{99}mTc, radiosilver-111, radiosilver-199, Actinium 225 and Bismuth^{213}.

Linkage of radioisotopes to antibodies may be performed with conventional bifunctional chelates. Since silver is monovalent, for radiosilver-111 and radiosilver-199 linkage, sulfur-based linkers may be used (Hazra et al., *Cell Biophys*, 24-25:1-7 (1994)). Linkage of silver radioisotopes may involved reducing the immunoglobulin with ascorbic acid. In another aspect, tiuxetan is an MX-DTPA linker chelator attached to ibritumomab to form ibritumomab tiuxetan (Zevalin) (Witzig, T.E, *Cancer Chemother Pharmacol*, 48 Suppl 1:S91-5 (2001). Ibritumomab tiuxetan can

react with radioisotypes such as indium^{111} (^{111}In) or ^{90}Y to form ^{111}In-ibritumomab tiuxetan and ^{90}Y-ibritumomab tiuxetan, respectively.

WHAT IS CLAIMED:

1. An isolated monoclonal antibody comprising a heavy chain amino acid having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 24, 25, 26, 27, 28, 29, 30, 71, 72, 73, 75, 77, 79, 90, 92, 94, 96, 98, 100, 102, 125, 128, 130, 133, 136, 137, 142, 146, 150, 153, 169, 171, 172 and 246 and wherein said monoclonal antibody specifically binds CA IX.

2. The antibody of claim 1, wherein said antibody is a fully human antibody.

3. The antibody of claim 1, wherein the antibody further comprises a light chain amino acid having an amino acid sequence selected from the group consisting of SEQ ID NOs: 32, 33, 34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 60, 61, 62, 63, 64, 66, 67, 68, 69, 81, 82, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 162, 168, 175, 177, 179 and 245.

4. The monoclonal antibody of claim 1, in association with a therapeutically acceptable carrier.

5. The monoclonal antibody of claim 1, wherein said antibody is conjugated to a therapeutic or cytotoxic agent.

6. The conjugate of claim 5 wherein the further therapeutic agent is a toxin.

7. The conjugate of claim 5 wherein the further therapeutic agent is a radioisotope.

8. A method of inhibiting cell proliferation associated with the expression of CA IX tumor antigen comprising treating cells expressing CA IX with an effective amount of an antibody of claim 1.

9. A method for treatment of a disease associated with the expression of CA IX in a patient, comprising administering to the patient an effective amount of an antibody of claim 1.

10. The method for treatment of claim 9 wherein the patient is a mammalian patient.

11. The method for treatment of claim 10 wherein the mammalian patient is human.

12. The method for treatment of claim 11 wherein the disease is a tumor.

13. The method for treatment of claim 12 wherein the tumor is selected from the group consisting of colorectal neoplasms, colorectal tumors, renal cell carcinoma (RCC), cervical carcinoma, cervical intraepithelial squamous and glandular neoplasia, esophageal tumors, and breast cancer.

14. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a heavy chain variable domain of a monoclonal antibody, wherein said nucleotide sequence is selected from the group consisting of SEQ ID NOs: 104, 106, 108, 110, 112, 114, 116, 118, 170, 172, 174, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211 and 212.

15. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a light chain variable domain of a monoclonal antibody, wherein said nucleotide sequence is selected from the group consisting of SEQ ID NOs: 105, 107, 109, 111, 113, 115, 117, 119, 176, 178, 180, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243 and 244.

Alignment of sequences using VH4-4

```
                                                CDR1                    CDR2
VH4-4          1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS SYYWS WIRQP AGKGL EWIGR IYTSG STNYN 60
MN-21.17.1_HC  1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS SYYWS WIRQP AGKGL EWIGR IYTSG STNYN 60
MN-22.19_HC    1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS FYYWN WIRQP AGKGL EWVGR IYTSG STNYN 60
MN-22.29_HC    1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS SYYWS WIRQP AGKGL EWIGR IYTSG STNYN 60
MN-22.3_HC     1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS SNYWS WIRQP AGKGL EWIGR IYTSG STNYN 60
MN-22.5_HC     1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS FYYWN WIRQP AGKGL EWVGR IYTSG STNYN 60

                 CDR2                                      CDR3
VH4-4          61-PSLKS RVTMS VDTSK NQFSL KLSSV TAADT AVYYC AR--- ----- ----- ----- ----- -  97  (SEQ ID NO:1)
MN-21.17.1_HC  61-PSLKS RVTMS VDTSK NQFSL KLSSV TAADT AVYYC ARDG- -QWLE DYGMD VWGQG TTVTV SS 119 (SEQ ID NO:2)
MN-22.19_HC    61-PSLKS RVTMS VDTSK NQFSL KLSSV TAADT AVYYC ARGGF LEWDY YYGMD VWGQG TTVTV SS 122 (SEQ ID NO:3)
MN-22.29_HC    61-PSLKS RVTMS VDTSK NQFSL KLSSV TAADT AVYYC ARDG- -QWLE DYGMD VWGQG TTVTV SS 119 (SEQ ID NO:4)
MN-22.3_HC     61-PSLKS RVTMS VDTSK NQFSL KLSSV TAADT AVYYC ARDQ- -GFLE WLPLD VWGQG TTVTV SS 119 (SEQ ID NO:5)
MN-22.5_HC     61-PSLKS RVTMS VDTSK NQFSL KLSSV TAADT AVYYC ARGGF LEWDY YYGMD VWGQG TTVTV SS 122 (SEQ ID NO:6)
```

FIG. 1

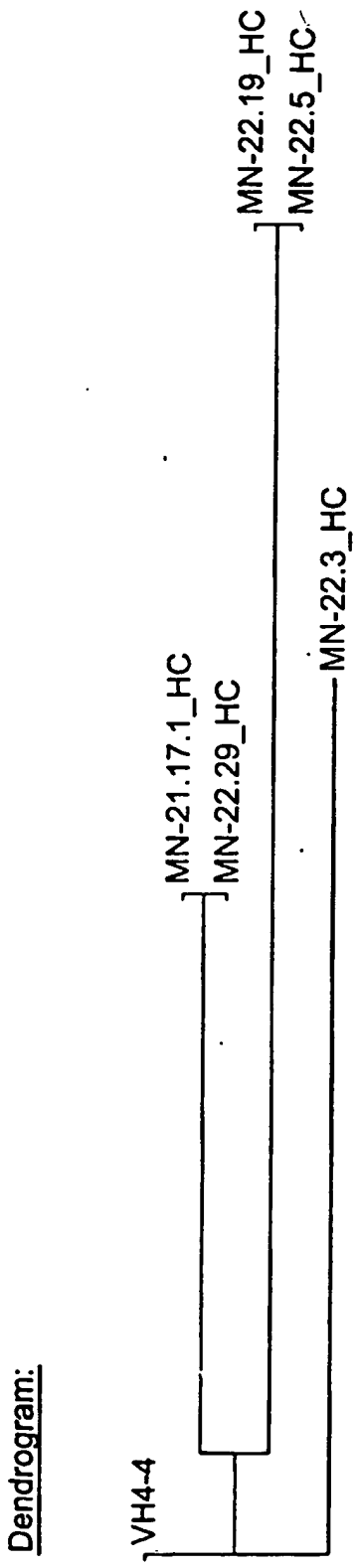

Dendrogram:
VH4-4
MN-21.17.1_HC
MN-22.29_HC
MN-22.3_HC
MN-22.19_HC
MN-22.5_HC

FIG. 2

Alignment of sequences using VH4-31

FIG.3A

		CDR1	CDR2
VH4-31	1-QVQLQ ESGPG LVKPS QTLSL TCTVS GGSIS SGGYY WSWIR QHPGK GLEWI GYIYY SGSTY 60		
MN-21.2.1_HC	1-QVQLQ ESGPG LVKPS QTLSL TCTVS GGSIS SGGYY WNWIR QHPGK GLEWI GYIYY SGSTY 60		
MN-21.5.2_HC	1-QVQLE QSGPG LVKPS QTLSL TCTVS GGSIS SGGRY WSWIR QHPGK GLEWI GYIYY SGSTY 60		
MN-21.6.1_HC	1-QVQLQ ESGPG LVKPS QTLSL TCTVS GGSIS SGGYY WSWIR QHPGK GLEWI GYIYY SGSTY 60		
MN-21.7.1_HC	1-QVQLQ ESGPG LVKPS QTLSL TCTVS GGSIS SGGYY WSWIR QHPGK GLEWI GYIYY SGNTY 60		
MN-21.9.1_HC	1-QVQLQ ESGPG LVKPS QTLSL TCTVS GGSIS SGGYY WSWIR QHPGK GLEWI GYIYY SGTTY 60		
MN-22.11_HC	1-QVQLQ ESGPG LVKPS QTLSL TCTVS GGSIS SGGYY WSWIR QHPGK GLEWI GYIYY SGSTY 60		
MN-22.15_HC	1-QVQLQ ESGPG LVKPS QTLSL TCTVS GGSIS SGGYY WSWIR QHPGK GLEWI GYIYY SGYTY 60		
MN-22.16_HC	1-QVQLQ ESGPG LVKPS QTLSL TCTVS GGSIS SGVYY WSWIR QHPGK GLEWI GYIYY SRSTY 60		
MN-22.18_HC	1-QVQLQ ESGPG LVKPS QTLSL TCTVS GGSIS SGGYY WNWIR QHPGK GLEWI GYIYY SGSTY 60		
MN-22.21_HC	1-QVQLQ ESGPG LVKPS QTLAL TCTVS GGSIS SGGYY WSWIR QHPGK GLEWI GYIYY SGYTY 60		
MN-22.23_HC	1-QVQLQ ESGPG LVKPS QTLSL TCTVS GGSIN SGGYY WSWIR QHPGK GLEWI GYIYY SGSTY 60		
MN-22.9_HC	1-QVQLQ ESGPG LVKPS QTLSL TCTVS GGSIN SGGYY WSWIR QHPGK GLEWI GYIYY SGSTY 60		

		CDR2	CDR3
VH4-31	61-YNPSL KSRVT ISVDT SKNQF SLKLS SVTAA DTAVY YCAR- ----- ----- ----- ----- 99		
MN-21.2.1_HC	61-YNPSL KSRVT ISVDT SKNQF SLKLS SVTAA DTAVY YCART YYD-- ILTGY PDAFD IWGQG 118		
MN-21.5.2_HC	61-YNPSL KSRVT ISIDT SKNQF SLKLS SVTAA DTAVY YCARA GTYYG ----S GSYLD YWGQG 116		
MN-21.6.1_HC	61-YNPSL KSRVT ISVDT SKNQF SLKLS SVTAA DTAVY YCARA GKYYG ----S GSYLD YWGQG 116		
MN-21.7.1_HC	61-YNPSL KSRIT ISVDT SKNQF SLKLS SVTAA DTAVY YCART YYD-- FLTGY PDAFD IWGQG 118		
MN-21.9.1_HC	61-YNPSL KSRVT ISVDT SKNQF SLKLS SVTAA DTAVY YCARV L---- LWFGE DYGVD VWGQG 116		
MN-22.11_HC	61-YNPSL KSRVT ISVDT SKNQF SLKLS SVTAA DTAVY YCARD GYNY- ----- -WYFD LWGRG 113		
MN-22.15_HC	61-YNPSL KSRVT ISVDT SKNQF SLKLS SVTAA DTAVY YCARD RYYDI LTGYY NYGMD VWGQG 120		
MN-22.16_HC	61-YNPSL KSRVT ISVDT SKNQF SLKLS SVTAA DTAVY YCARG N---- -AHDY YFGMD VWGQG 115		
MN-22.18_HC	61-YNPSL KSRVT ISVDT SKNQF SLKLS SVTAA DTAVY YCART YYD-- ILTGY PDAFD IWGQG 118		
MN-22.21_HC	61-YNPSL KSRVT ISVDT SKNQF SLKLS SVTAA DTAVY YCARE T---- VTDYY YYGMD VWGQG 116		
MN-22.23_HC	61-YNPSL KSRVI ISVDT SENQF SLKLS SVTAA DTAVY YCARE R---- VTDYY YYGLD VWGQG 116		
MN-22.9_HC	61-YNPSL KSRVI ISVDT SENQF SLKLS SVTAA DTAVY YCARE R---- VTDYY YYGLD VWGQG 116		

VH4-31	------	-	99	(SEQ ID NO:7)
MN-21.2.1_HC	119-TMVTVS	S	125	(SEQ ID NO:8)
MN-21.5.2_HC	117-TLVTVS	S	123	(SEQ ID NO:9)
MN-21.6.1_HC	117-TLVTVS	S	123	(SEQ ID NO:10)
MN-21.7.1_HC	119-TMVTVS	S	125	(SEQ ID NO:11)
MN-21.9.1_HC	117-TTVTVS	S	123	(SEQ ID NO:12)
MN-22.11_HC	114-TLVTVS	S	120	(SEQ ID NO:13)
MN-22.15_HC	121-TTVTVS	S	127	(SEQ ID NO:14)
MN-22.16_HC	116-TTVTVS	S	122	(SEQ ID NO:15)
MN-22.18_HC	19-TMVTVS	S	125	(SEQ ID NO:16)
MN-22.21_HC	117-TTVTVS	S	123	(SEQ ID NO:17)
MN-22.23_HC	117-TTVTVS	S	123	(SEQ ID NO:18)
MN-22.9_HC	117-TTVTVS	S	123	(SEQ ID NO:19)

FIG. 3B

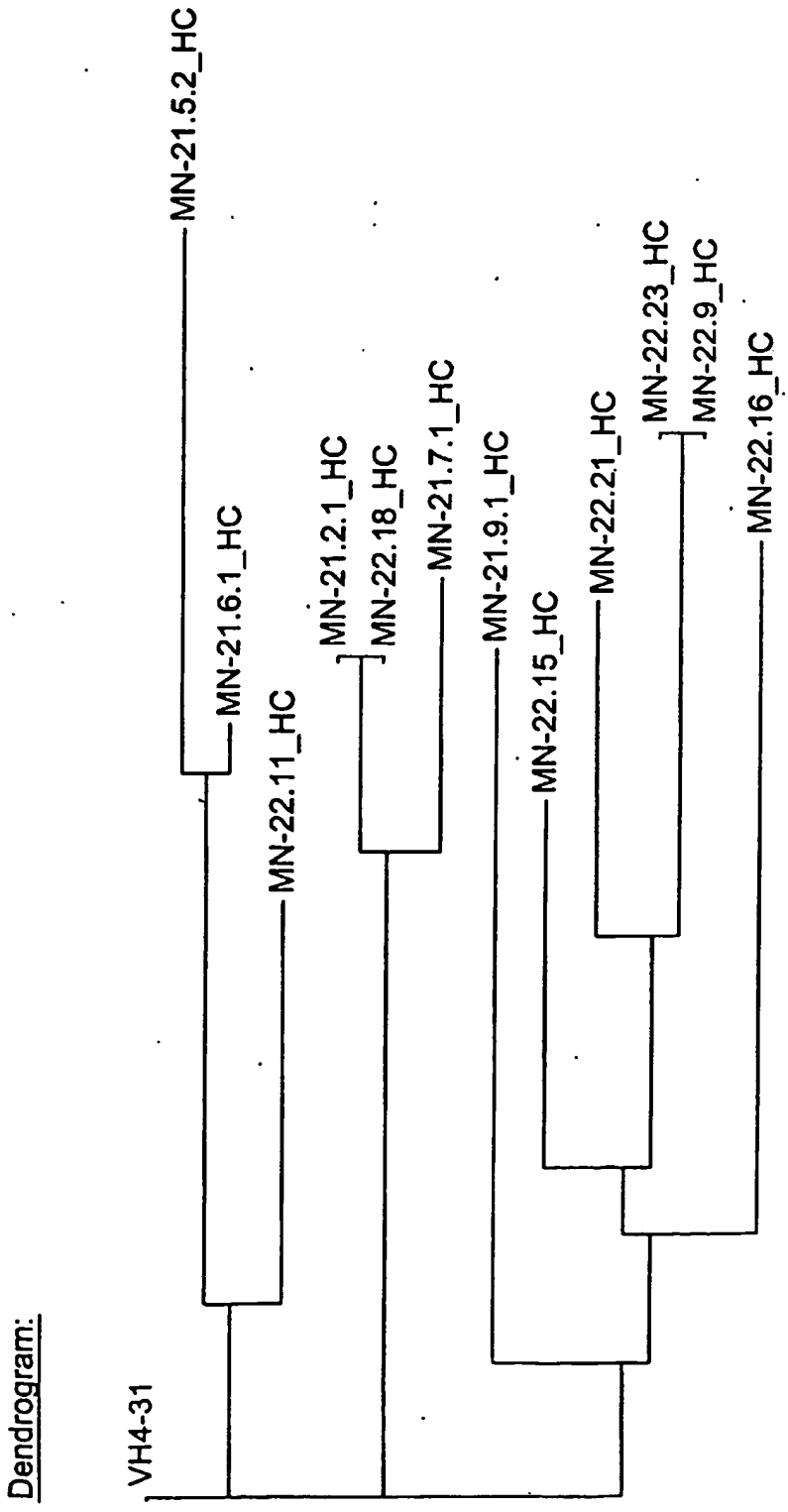

Dendrogram:
VH4-31
MN-21.5.2_HC
MN-21.6.1_HC
MN-22.11_HC
MN-21.2.1_HC
MN-22.18_HC
MN-21.7.1_HC
MN-21.9.1_HC
MN-22.15_HC
MN-22.21_HC
MN-22.23_HC
MN-22.9_HC
MN-22.16_HC

FIG. 4

Alignment of sequences using VH4-39

```
                                        CDR1                    CDR2
VH4-39          1-QLQLQ ESGPG LVKPS ETLSL TCTVS GGSIS SSSYY WGWIR QPPGK GLEWI GSIYY SGST 59
MN-21.10.1_HC   1---QLE QSGPG LVKPS ETLSL TCTVS DGSIS SSSYY WGWIR QPPGK GLEWI GSIYY SGST 57
MN-22.25_HC     1-QLQLQ ESGPG LVKPS ETLSL TCTVS DGSIS SSSYY WGWIR QPPGK GLEWI GSIYY SGST 59

                    CDR2                                CDR3
VH4-39          60-YYNPS LKSRV TISVD TSKNQ FSLKL SSVTA ADTAV YYCAR ----- ----- ----- ---  99 (SEQ ID NO:20)
MN-21.10.1_HC   58-YYNPS LKSRV TISVD TSKNQ FSLKL SSVTA ADTAV YYCAR HGSFF DYWGQ GTLVT VSS 115 (SEQ ID NO:21)
MN-22.25_HC     60-YYNPS LKSRV TISVD TSKNQ FSLKL SSVTA ADTAV YYCAR HGSFF DYWGQ GTLVT VSS 117 (SEQ ID NO:22)
```


FIG. 5

Alignment of sequences using VH4-59

```
                                                CDR1                                CDR2
VH4-59          1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS SYYWS WIRQP PGKGL EWIGY IYYSG STNY 59
MN-21.1.1_HC    1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS SYYWS WIRQP PGKGL EWIGY IYYSG STNY 59
MN-21.14.1_HC   1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS SYYWS WIRQP PGKGL EWIGY IYYSG STNY 59
MN-22.12_HC     1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS SYYWS WIRQP PGKGL EWIGY IYSSG STNY 59
MN-22.17_HC     1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS SYYWS WIRQP PGKGL EWIGY IYYSG STNY 59
MN-22.26_HC     1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS RNYWS WIRQP PGKGL EWIGY IYYSG STKY 59
MN-22.27_HC     1-QVQLQ ESGPG LVKPS ETLSL TCTVS GGSIS SYYWS WIRQP PGKGL EWIGY IYYSG STNY 59
MN-22.8.1_HC    1------ -SGPG LVKPS ETLSL TCTVS GGSIS SYYWS WIRQP PGKGL EWIGY IYYSG STNY 53

                CDR2                                                CDR3
VH4-59          60-NPSLK SRVTI SVDTS KNQFS LKLSS VTAAD TAVYY CAR-- ----- ----- ----- -----  97
MN-21.1.1_HC    60-NPSLK SRVTI SVDTS KNQFS LKLSS VTAAD TAVYY CARRG YDILT GYDY- -FDYW GQGTL 117
MN-21.14.1_HC   60-NPSLK SRVTI SVDTS KNQFS LKLSS VTAAD TAVYY CARRG YDFLT GYDY- -FDYW GQGTL 117
MN-22.12_HC     60-NPSLK SRVTI SVDTS KNQFS LKLSS VTAAD TAVYY CARDQ HSSSF YYYYY GMDVW GQGTT 119
MN-22.17_HC     60-NPSLK SRVTI SVDTS KNQFS LKLSS VTAAD TAVYY CARRG YDILT GYDY- -FDYW GQGTL 117
MN-22.26_HC     60-NPSLK SRVTI SVDTS KNQFS LKLSS VTAAD TAVYY CARSY YDFLT GYYHV -FDYW GQGTL 118
MN-22.27_HC     60-NPSLK SRVTI SVDTS KNQFS LKLSS VTAAD TAVYY CARRG YDFLT GYDY- -FDYW GQGTL 117
MN-22.8.1_HC    54-NPSLK SRVTI SVDTS KNQFS LKLTS VTAAD TAVYY CARDQ HSSSV YYYYY GMDVW GQGTT 113

VH4-59          97-------  97 (SEQ ID NO:23)
MN-21.1.1_HC    118-VTVSS 122 (SEQ ID NO:24)
MN-21.14.1_HC   118-VTVSS 122 (SEQ ID NO:25)
MN-22.12_HC     120-VTVSS 124 (SEQ ID NO:26)
MN-22.17_HC     118-VTVSS 122 (SEQ ID NO:27)
MN-22.26_HC     119-VTVSS 123 (SEQ ID NO:28)
MN-22.27_HC     118-VTVSS 122 (SEQ ID NO:29)
MN-22.8.1_HC    114-VTVSSA 119 (SEQ ID NO:30)
```

FIG. 6

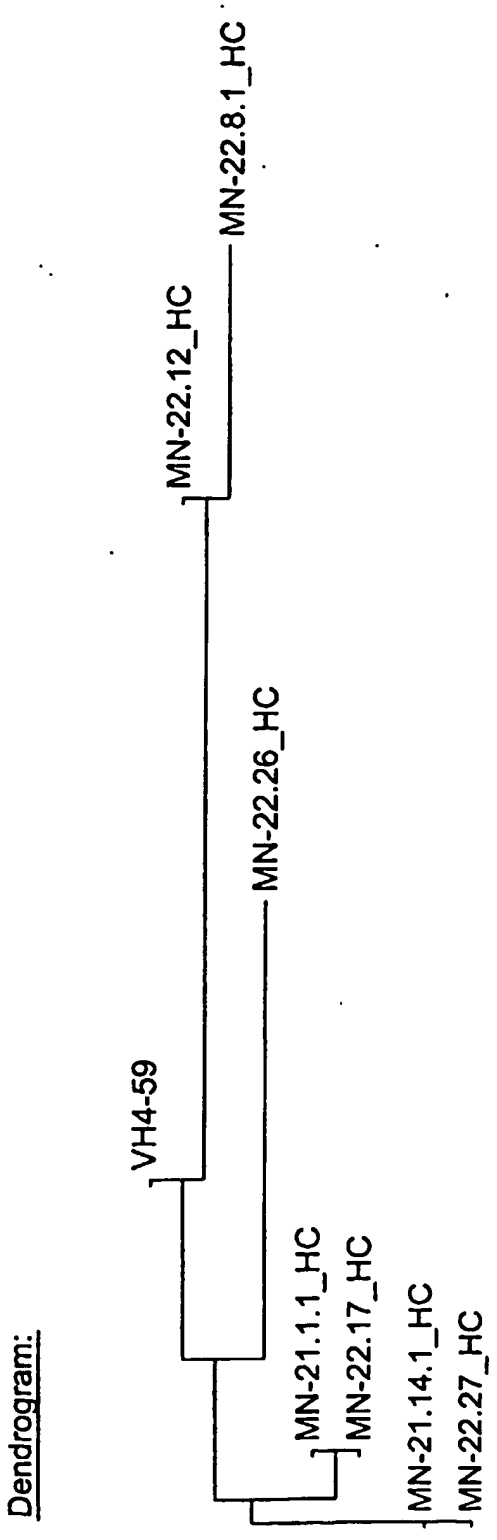

Dendrogram:
VH4-59
MN-21.1.1_HC
MN-22.17_HC
MN-21.14.1_HC
MN-22.27_HC
MN-22.26_HC
MN-22.12_HC
MN-22.8.1_HC

FIG. 7

Alignment of sequences using VK-A19

		CDR1 CDR2
A19	1-DIVMT QSPLS LPVTP GEPAS ISCRS SQSLL HSNGY NYLDW YLQKP GQSPQ LLIYL GSNRA	60
MN-21.5.2_LC	1-DIVMT QSPLS LPVTP GEPAS ISCRS SQSLL HSNGY NYLDW YLQKP GQCPQ LLIYL GSNRA	60
MN-21.6.1_LC	1-DIVMT QSPLS LPVTP GEPAS ISCRS SQSLL HSNGY NYLDW YLQKP GQSPQ LLIYL GSNRA	60
MN-22.7_LC	1-DIVMT QSPLS LPVTP GEPAS ISCRS SQSLL YSNGY NYLDW YLQKP GQSPQ FLIYL GSNRA	60
	CDR2 CDR3	
A19	61-SGVPD RFSGS GSGTD FTLKI SRVEA EDVGV YYCMQ ALQTP ----- ----- --	100 (SEQ ID NO:31)
MN-21.5.2_LC	61-SGVPD RFSGS GSGTD FTLKI SRVEA EDVGV YYCMQ ALQTP LTFGG GTKVE IK	112 (SEQ ID NO:32)
MN-21.6.1_LC	61-SGVPD RFSGS GSGTD FTLKI SRVEA EDVGV YYCMQ ALQTP LTFGG GTKVE IK	112 (SEQ ID NO:33)
MN-22.7_LC	61-SGVPD RFSGS GSGTD FTLKI SRVEA EDVGV YYCMQ ALQTP RSFGQ GTKLE IK	112 (SEQ ID NO:34)

FIG. 8

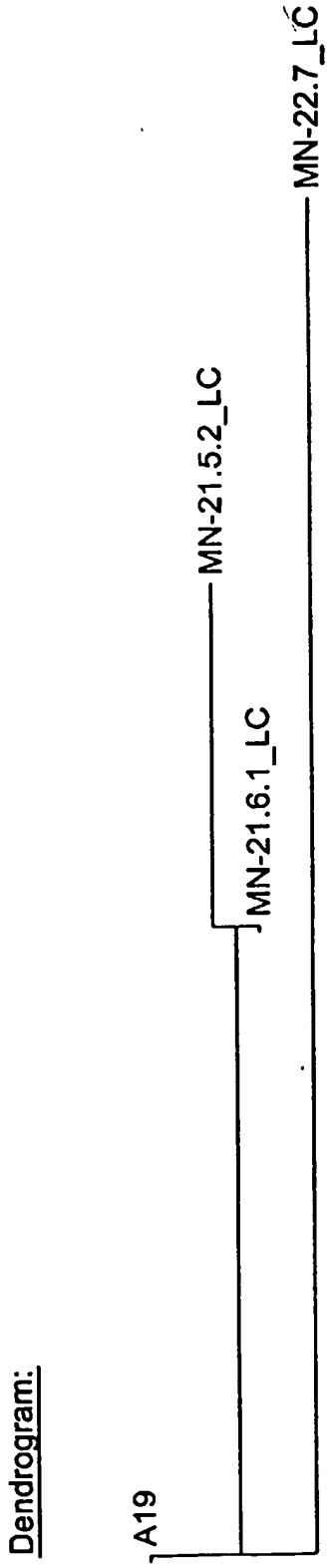


FIG. 9

Alignment of sequences using VK-A27

FIG. 10

		CDR1	CDR2	
A27	1-EIVLT QSPGT LSLSP GERAT LSCRA SQSVS SSYLA WYQQK PGQAP RLLIY GASSR ATGIP	60		
MN-22.8.1_LC	1-ETQLT QSPGT LSLSP GERAT LSCRA SQSIY INYLA WYQQK PGQAP RLLIS GASSR ATGIP	60		
MN-21.17.1_LC	1-EIVLT QSPGT LSLSP GERAT LSCWA SQSVF SNYLA WYQQK PGQAP RLLIY GASSR ATGIP	60		
MN-21.8.1_LC	1-EIVLT QSPGT LSLSP GERAT LSCRA SQSVS SSYLA WYQQK PGQAP RLLIY GASSR ATGIP	60		
MN-22.11_LC	1-EIVLT QSPGT LSLSP GERAT LSCRA SQSVG SSYLA WYQQK PGQTP RLLIY GASSR ATGIP	60		
MN-22.19_LC	1-EIVLT QSPGT LSLSP GERAT LSCRA SQSVS VTYLA WYQQK PGQAP RLLIY GASSR ATGIP	60		
MN-22.26_LC	1-EIVLT QSPGT LSLSP GERAT LSCRA SQSVY SNYFA WYQQK PGQAP RLLIY GASSR AAGIP	60		
MN-22.27_LC	1-EIVLT QSPGT LSLSP GERAT LSCWA SQSVF SNYLA WYQQK PGQAP RLLIY GASSR ATGIP	60		
MN-22.3_LC	1-EIVLT QSPGT LSLSP GERAT LSCRA SQSVS SYYLA WYQQK PGQAP RLLIY GASSR ATGIP	60		
MN-22.4_LC	1-EIVLT QSPGT LSLSP GERAT LSCRA SQSVS SSYLA WYQQK PGQAP RLLIY GASSR ATGIP	60		
MN-22.5_LC	1-EIVLT QSPGT LSLSP GERAT LSCRA SQSVS VTYLA WYQQK PGQAP RLLIY GASSR ATGIP	60		
MN-22.9_LC	1-EIVLT QSPGT LSLSP GERAT LSCRA SQSVY SNYFA WYQQK PGQAP RLLIY GASSR AAGIP	60		

		CDR3		
A27	61-DRFSG SGSGT DFTLT ISRLE PEDFA VYYCQ QYGSS P---- ----- --- -	96	(SEQ ID NO:35)	
MN-22.8.1_LC	61-DRFSG SGSGT DFTLT ISRLE PEDFA VYYCQ QYGRS L--TF GGGTE VEIKR	108	(SEQ ID NO:36)	
MN-21.17.1_LC	61-DRFSG SGSGT DFPLT ISRLE PEDFA VYYCQ QYGSS P-LTF GGGTK VEIK	108	(SEQ ID NO:37)	
MN-21.8.1_LC	61-DRFSG SGSGT DFTLT ISRLE PEDFA VYSCQ QYGSS L--TF GGGTK VEIK	107	(SEQ ID NO:38)	
MN-22.11_LC	61-DRFSG SGSGT DFTLT ISRLE PEDFA VYYCQ QYGFS P-LTF GGGTK VEIK	108	(SEQ ID NO:39)	
MN-22.19_LC	61-DRFSG SGSGT DFTLT ISRLE PEDFA VYYCQ QYGSS V--TF GGGTK VEIK	107	(SEQ ID NO:40)	
MN-22.26_LC	61-DRFSG SGSGT DFTLT ISRLE PEDFA VYYCQ QYGTS PRFSF GQGTK LEIK	109	(SEQ ID NO:41)	
MN-22.27_LC	61-DRFSG SGSGT DFPLT ISRLE PEDFA VYYCQ QYGSS P-LTF GGGTK VEIK	108	(SEQ ID NO:42)	
MN-22.3_LC	61-DRFSG SGSGT DFTLT ISRLE PEDFA VYYCQ QYGTS P-ITF GQGTR LEIK	108	(SEQ ID NO:43)	
MN-22.4_LC	61-DRFSG SGSGT DFTLT ISRLE PEDFA VYSCQ QYGSS L--TF GGGTK VEIK	107	(SEQ ID NO:44)	
MN-22.5_LC	61-DRFSG SGSGT DFTLT ISRLE PEDFA VYYCQ QYGSS V--TF GGGTK VEIK	107	(SEQ ID NO:45)	
MN-22.9_LC	61-DRFSG SGSGT DFTLT ISRLE PEDFA VYYCQ QYGTS PRFSF GQGTK LEIK	109	(SEQ ID NO:46)	

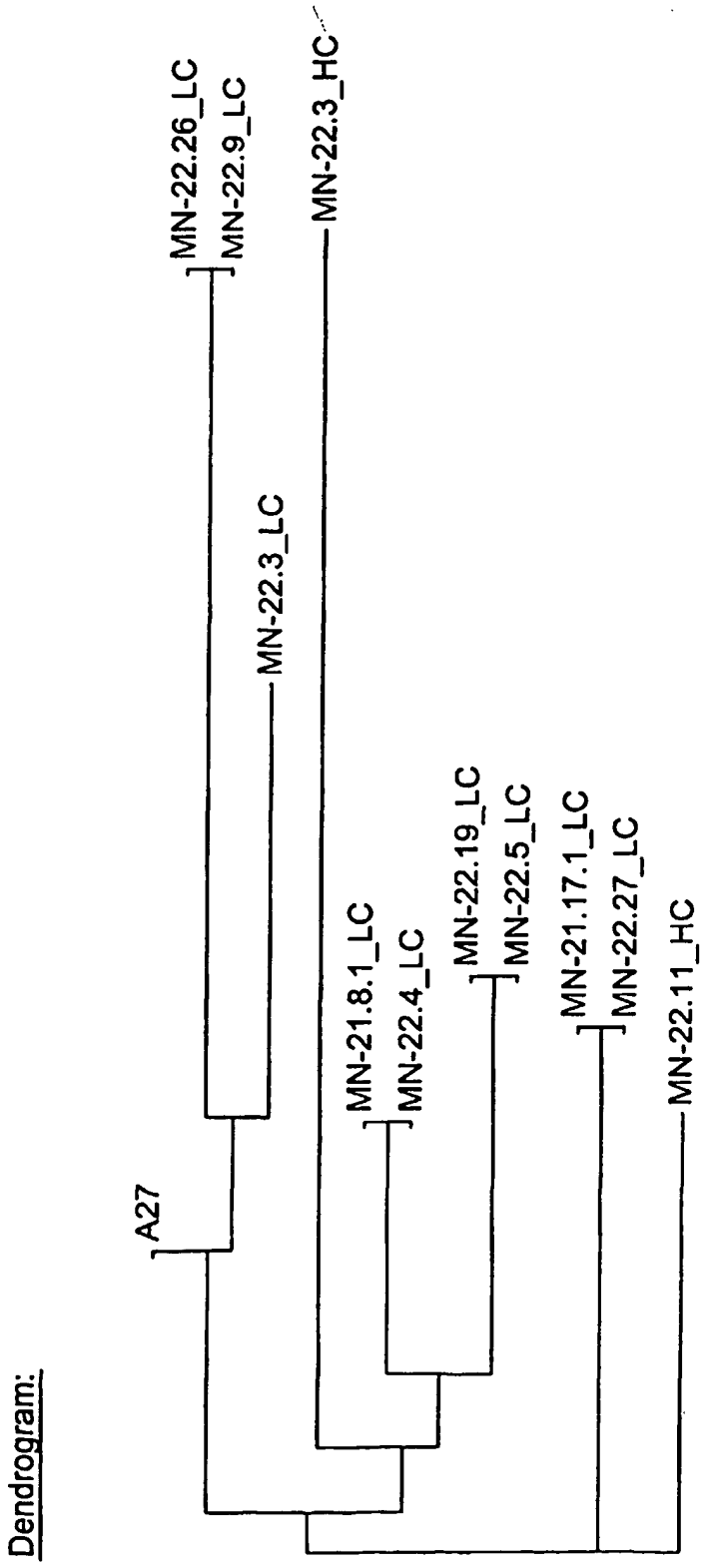

Dendrogram:
A27
MN-22.26_LC
MN-22.9_LC
MN-22.3_LC
MN-22.3_HC
MN-21.8.1_LC
MN-22.4_LC
MN-22.19_LC
MN-22.5_LC
MN-21.17.1_LC
MN-22.27_LC
MN-22.11_HC

FIG. 11

Alignment of sequences using VK-A30

FIG. 12

		CDR1	CDR2
A30	1-DIQMT QSPSS LSASV GDRVT ITCRA SQGIR NDLGW YQQKP GKAPK RLIYA ASSLQ SGVPS 60		
MN-21.2.1_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQDIR NDLGW YQQKP GKAPK RLICA ASSLQ SGVPS 60		
MN-21.7.1_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQAIR NDLGW YQQKP GKAPK RLIYA ASSLQ SGVPS 60		
MN-22.10_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQGIR YDLGW YQQKP GKAPK RLIYA TSNLQ SGVPS 60		
MN-22.14_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQGIR NDLGW YQQKP GKAPK RLIYA ASSLQ SGVPS 60		
MN-22.18_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQDIR NDLGW YQQKP GKAPK RLICA ASSLQ SGVPS 60		
MN-22.20_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQGIR NDLGW YQQKP GKAPK RLIYA ASSLQ SGVPS 60		
MN-22.21_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQAIR NDLGW YQQKP GKAPK RLIYA ASSLQ SGVPS 60		
MN-22.24_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQGIR YDLGW YQQKP GKAPK RLIYA TSNLQ SGVPS 60		
MN-22.25_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQGIR NDLGW YQQKP GKAPK RLIYA ASSLQ SGVPS 60		
MN-22.28.1_LC	1-DIRLT QSPSS LSASV GDRVT ITCRA SQGIR NDLGW YQQKP GKAPK RLIYA ASSLQ SGVPS 60		
MN-22.30_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQGIR NDLGW YQQKP GKAPK RLIYA ASSLQ SGVPS 60		

		CDR3
A30	61-RFSGSG SGTEF TLTIS SLQPE DFATY YCLQH NSYP- ----- ----- - 94 (SEQ ID NO:47)	
MN-21.2.1_LC	61-RFSGSG SGTEF TLTIS SLQPE DFATY YCLQH NSYPW TFGQG TKVEI K 107 (SEQ ID NO:48)	
MN-21.7.1_LC	61-RFSGSG SGSEF TLTIS SLQPE DFATY YCLQY HSYPW TFGQG TKVEI K 107 (SEQ ID NO:49)	
MN-22.10_LC	61-RFSGSG SGTEF TLTIS SLQPE DFATY YCLQH NSYPP TFGPG TKVDI K 107 (SEQ ID NO:50)	
MN-22.14_LC	61-RFSGSG SGTEF TLTIS SLQPE DFATY YCLQH NSYPL TFGGG TKVEI K 107 (SEQ ID NO:51)	
MN-22.18_LC	61-RFSGSG SGTEF TLTIS SLQPE DFATY YCLQH NSYPW TFGQG TKVEI K 107 (SEQ ID NO:52)	
MN-22.20_LC	61-RFSGSG SGTEF TLTIS SLQPE DFTTY YCLQY KSYPW TFGQG TKVEI K 107 (SEQ ID NO:53)	
MN-22.21_LC	61-RFSGSG SGSEF TLTIS SLQPE DFATY YCLQY HSYPW TFGQG TKVEI K 107 (SEQ ID NO:54)	
MN-22.24_LC	61-RFSGSG SGTEF TLTIS SLQPE DFATY YCLQH NSYPP TFGPG TKVDI K 107 (SEQ ID NO:55)	
MN-22.25_LC	61-RFSGSG SGTEF TLTIS SLQPE DFTTY YCLQY KSYPW TFGQG TKVEI K 107 (SEQ ID NO:56)	
MN-22.28.1_LC	61-RFSGSG SGTEF TLTIS SLQPE DFATY YCLQH NSYPL TFGGG TKVEI K 107 (SEQ ID NO:57)	
MN-22.30_LC	61-RFSGSG SGTDF TLTIS SLQPE DFATY YCLQH YSYPL TFGGG TKVEI K 107 (SEQ ID NO:58)	

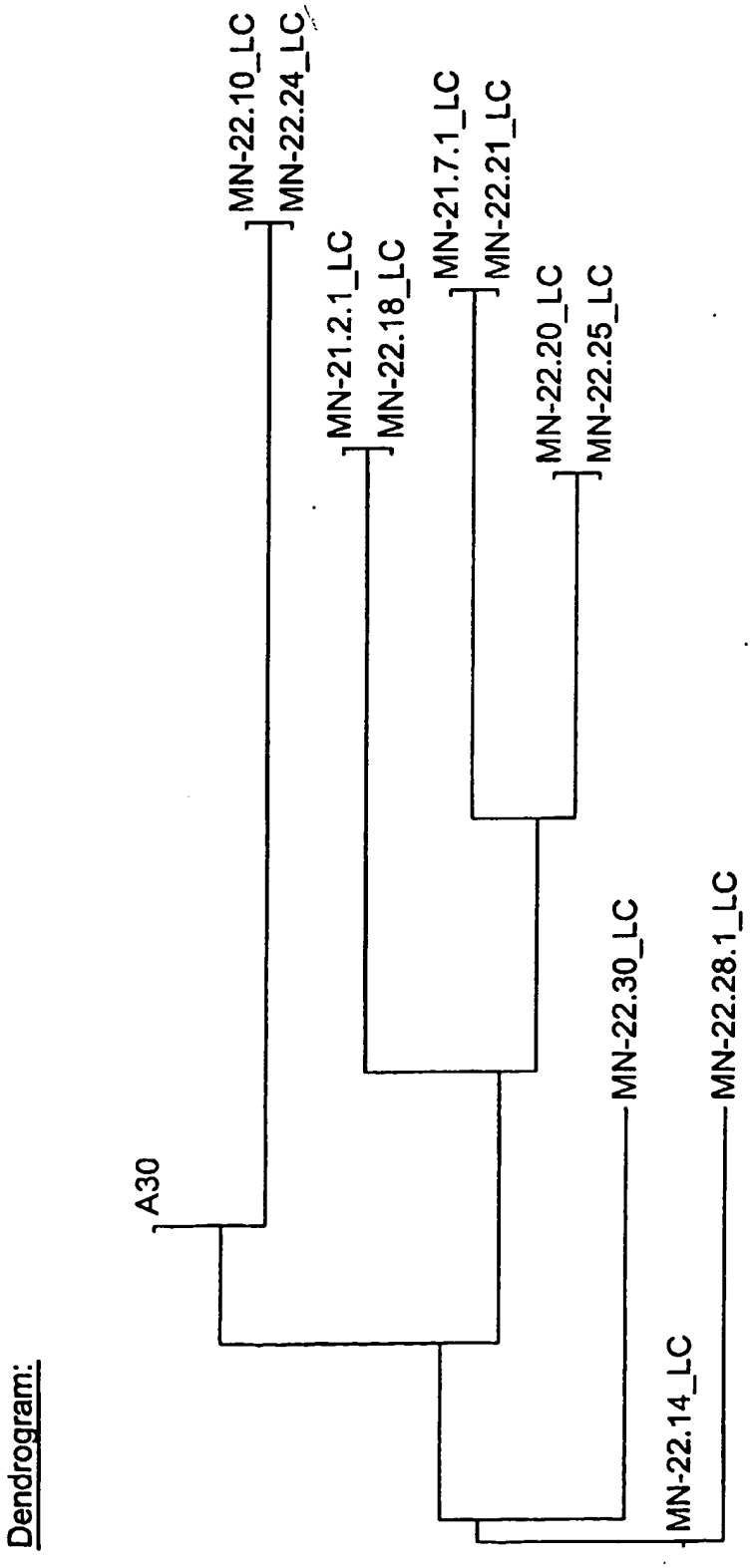

Dendrogram:
A30
MN-22.10_LC
MN-22.24_LC
MN-21.2.1_LC
MN-22.18_LC
MN-21.7.1_LC
MN-22.21_LC
MN-22.20_LC
MN-22.25_LC
MN-22.30_LC
MN-22.14_LC
MN-22.28.1_LC

FIG. 13

Alignment of sequences using VK-L5

```
                                        CDR1                                CDR2
L5              1-DIQMT QSPSS VSASV GDRVT ITCRA SQGIS SWLAW YQQKP GKAPK LLIYA ASSLQ SGVPS 60
MN-21.1.1_LC    1-DIQMT QSPSS VSASV GDRVT ITCRA SQGIS IWLTW YQQKP GKAPK LLIYA ASSLQ SGVPS 60
MN-21.14.1_LC   1-DIQMT QSPSS VSASV GDRVT ITCRA SQGIS SWLAW FQQKP GKAPK LLIYA ASSLQ SGVPS 60
MN-22.12_LC     1-DIQMT QSPSS VSASV GDRVT ITCRA SQGIS SWLAW YQQKP GKAPK LLIYA ASSLQ SGVPS 60
MN-22.17_LC     1-DIQMT QSPSS VSASV GDRVT ITCRA SQGIS IWLTW YQQKP GKAPK LLIYA ASSLQ SGVPS 60
MN-22.29_LC     1-DIQMT QSPSS VSASV GDRVT ITCRA SQGIS SWLAW FQQKP GKAPK LLIYA ASSLQ SGVPS 60

                                        CDR3
L5              61-RFSGS GSGTD FTLTI SSLQP EDFAT YYCQQ ANSFP ----- ----- --  95 (SEQ ID NO:59)
MN-21.1.1_LC    61-RFSGS GSGTD FTLTI SSLQP EDFAT YYCQQ ANSFP ITFGQ GTRLE IK 107 (SEQ ID NO:60)
MN-21.14.1_LC   61-RFSGS GSGTD FTLTI SSLQP EDFAT YYCQQ ANSFP ITFGQ GTRLE IK 107 (SEQ ID NO:61)
MN-22.12_LC     61-RFSGS GSGTD FTLTI SSLQP EDFAT YYCQQ ANSFP LTFGP GTKVD IK 107 (SEQ ID NO:62)
MN-22.17_LC     61-RFSGS GSGTD FTLTI SSLQP EDFAT YYCQQ ANSFP ITFGQ GTRLE IK 107 (SEQ ID NO:63)
MN-22.29_LC     61-RFSGS GSGTD FTLTI SSLQP EDFAT YYCQQ ANSFP ITFGQ GTRLE IK 107 (SEQ ID NO:64)
```

FIG. 14

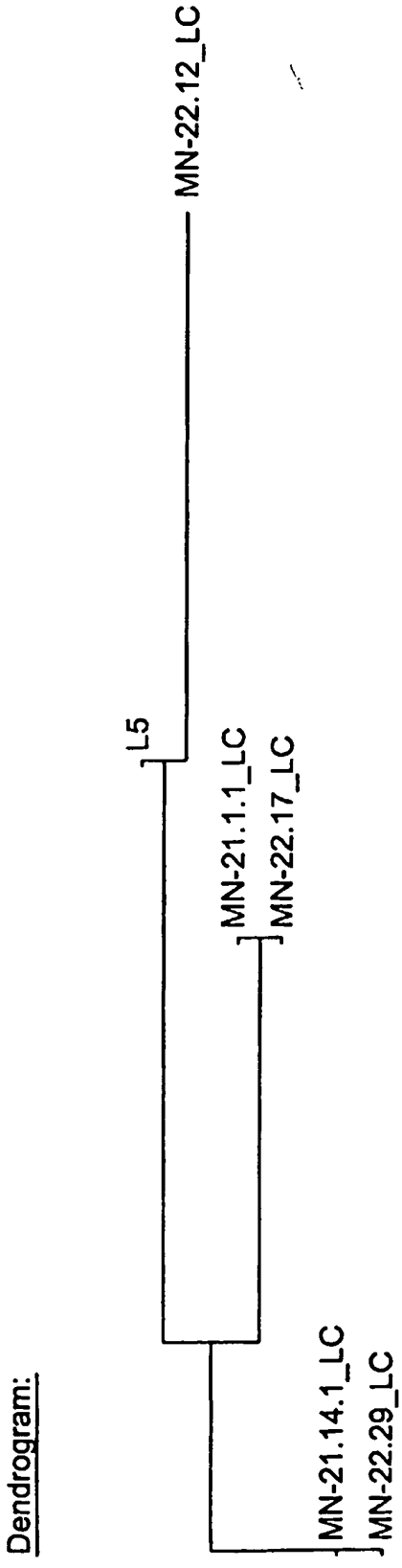

Dendrogram:
MN-21.14.1_LC
MN-22.29_LC
MN-21.1.1_LC
MN-22.17_LC
L5
MN-22.12_LC

FIG. 15

ignment of sequences using VK-012

		CDR1 CDR2
012	1-DIQMT QSPSS LSASV GDRVT ITCRA SQSIS SYLNW YQQKP GKAPK LLIYA ASSLQ SGVPS	60
MN-21.9.1_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQSIT NYLNW YQQKP GKAPK LLIYA ASSLQ SGVPS	60
MN-22.15_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQSIS SYLNW YQQKP GKVPK LLIYV ASSLQ SGVPS	60
MN-22.16_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQSIS SYLNW YQQKP GKVPK LLIYV ASSLQ SGVPS	60
MN-22.23_LC	1-DIQMT QSPSS LSASV GDRVT ITCRA SQSIT NYLNW YQQKP GKAPK LLIYA ASSLQ SGVPS	60
		CDR3
012	61-RFSGS GSGTD FTLTI SSLQP EDFAT YYCQQ SYSTP ----- ----- --	95 (SEQ ID NO:65)
MN-21.9.1_LC	61-RFSGS GSGTD FTLTI SSLQP EDFAT YYCQQ SYITP LTFGG GTEVE IK	107 (SEQ ID NO:66)
MN-22.15_LC	61-RFSGS GSGTD FTLTI SSLQP EDFAT YYCQQ SYSTP LTFGG GTKVE IK	107 (SEQ ID NO:67)
MN-22.16_LC	61-RFSGS GSGTD FTLTI SSLQP EDFAT YYCQQ SYSTP LTFGG GTKVE IK	107 (SEQ ID NO:68)
MN-22.23_LC	61-RFSGS GSGTD FTLTI SSLQP EDFAT YYCQQ SYITP LTFGG GTEVE IK	107 (SEQ ID NO:69)

FIG. 16

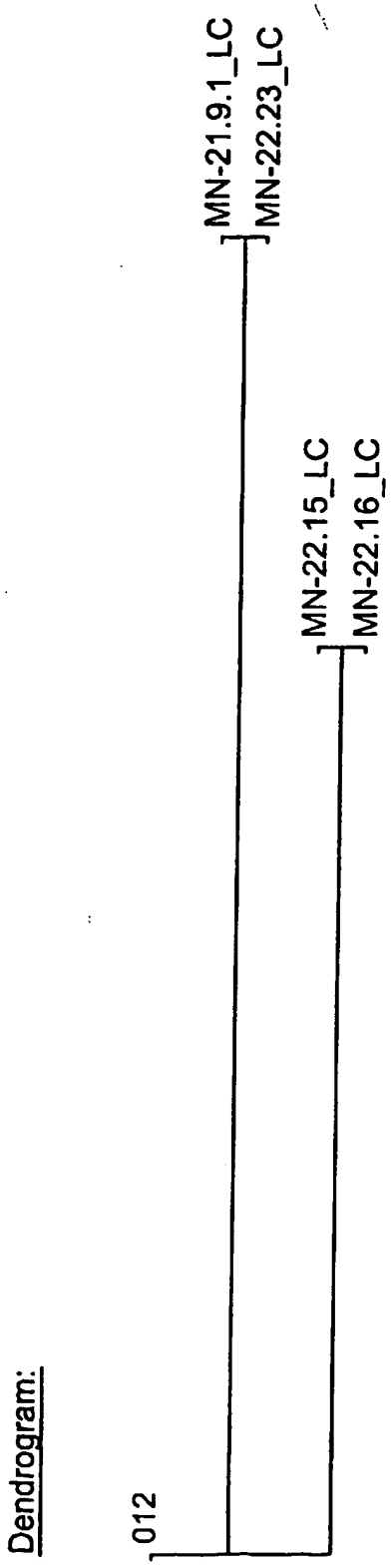


FIG. 17

Well	Single Cell	Vgamma/D/J	FR1	CDR1	FR2	CDR2
-	-	Germline	QVQLQESGPGLVKPSETLSLTCTVSGGSIS	SYYWS	WIRQPPGKGLEWIG	YIYYSGSTNYNPSLKS
43G10	80	VH4-59/D3-3/JH6b	----------	-----	----------	----------
44D2	141		----------S-------	-----	----------	----------
41D1	174		----------	-----	----------	--F-------
-	-	Germline	QVQLQESGPGLVKPSQTLSLTCTVSGGSIS	SGGYYWS	WIRQHPGKGLEWIG	YIYYSGSTYYNPSLKS
45C9	23	VH4-30.1/D3-9/JH5b	----------	-------	----------	----------
-	-	Germline	EVQLVESGGGLVKPGGSLRLSCAASGFTFS	SYSMN	WVRQAPGKGLEWVS	SISSSSSYIYYADSVKG
49C8	51	VH3-21/D5-18/JH4b	------F----------	-FR--	-------E------	--T----------
-	-	Germline	QVQLQESGPGLVKPSETLSLTCTVSGGSIS	SYYWS	WIRQPAGKGLEWIG	RIYTSGSTNYNPSLKS
42E4	109	VH4-04/D1-20/JH6b	----------	-----	----------	----------

Well	Single Cell	Vgamma/D/J	FR3	CDR3	FR4	
-	-	Germline	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR		WGQGTTVTVSS	SEQ ID NO: 70
43G10	80	VH4-59/D3-3/JH6b	----------	DTRTIFGVVSGMDV	----------	SEQ ID NO: 71
44D2	141		---------N-H----------	DTRTIFGVVSGMDV	----------	SEQ ID NO: 72
41D1	174		----------	DTRTIFGVVSGMDV	----------	SEQ ID NO: 73
-	-	Germline	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR		WGQGTLVTVSS	SEQ ID NO: 74
45C9	23	VH4-30.1/D3-9/JH5b	----------	ENYDILTGFNWFDP	----------	SEQ ID NO: 75
-	-	Germline	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR		WGQGTLVTVSS	SEQ ID NO: 76
49C8	51	VH3-21/D5-18/JH4b	----------	FTAMALDY	----------	SEQ ID NO: 77
-	-	Germline	RVTMSVDTSKNQFSLKLSSVTAADTAVYYCAR		WGQGTTVTVSS	SEQ ID NO: 78
42E4	109	VH4-04/D1-20/JH6b	----------	LITGPYGMDV	----------	SEQ ID NO: 79

FIG. 18

Well	Single Cell	Vkappa/J	FR1	CDR1	FR2	CDR2
-	-	Germline	EIVLTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT
43G10	80	A27/JK4	----------	------Y-----	----------	------P
44D2	141		----------	-T--T-Y-T---	----------	------
41D1	174		----------	------Y-----	----------	------
-	-	Germline	DIQMTQSPSSVSASVGDRVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	AASSLQS
45C9	23	L19/JK1	----------	----------	----------V---	ST-R---
-	-	Germline	DIQMTQSPSSLSASVGDRVTITC	RASQGISNYLA	WYQQKPGKVPKLLIY	AASTLQS
49C8	51	A20/JK1	----------	------F----	----------	-------
-	-	Germline	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS
42E4	109	02/JK3	----------	----------	----------	T--N-RG

Well	Single Cell	Vkappa/J	FR3	CDR3	FR4	
-	-	Germline	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSLT	FGGGTKVEIK	SEQ ID NO: 80
43G10	80	A27/JK4	----------	-H--R---	----------	SEQ ID NO: 81
44D2	141		----------F--	-H--R---	----------	SEQ ID NO: 82
41D1	174		----------D-------	-H--R---	----------	SEQ ID NO: 83
-	-	Germline	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFWT	FGQGTKVEIK	SEQ ID NO: 84
45C9	23	L19/JK1	----------	---D--R-	----------	SEQ ID NO: 85
-	-	Germline	GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC	QKYNSAPWT	FGQGTKVEIK	SEQ ID NO: 86
49C8	51	A20/JK1	----------V---	L--------	----------	SEQ ID NO: 87
-	-	Germline	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYSTPFT	FGPGTKVDIK	SEQ ID NO: 88
42E4	109	02/JK3	----------G----------	-----SL--	----------	SEQ ID NO: 89

FIG. 19

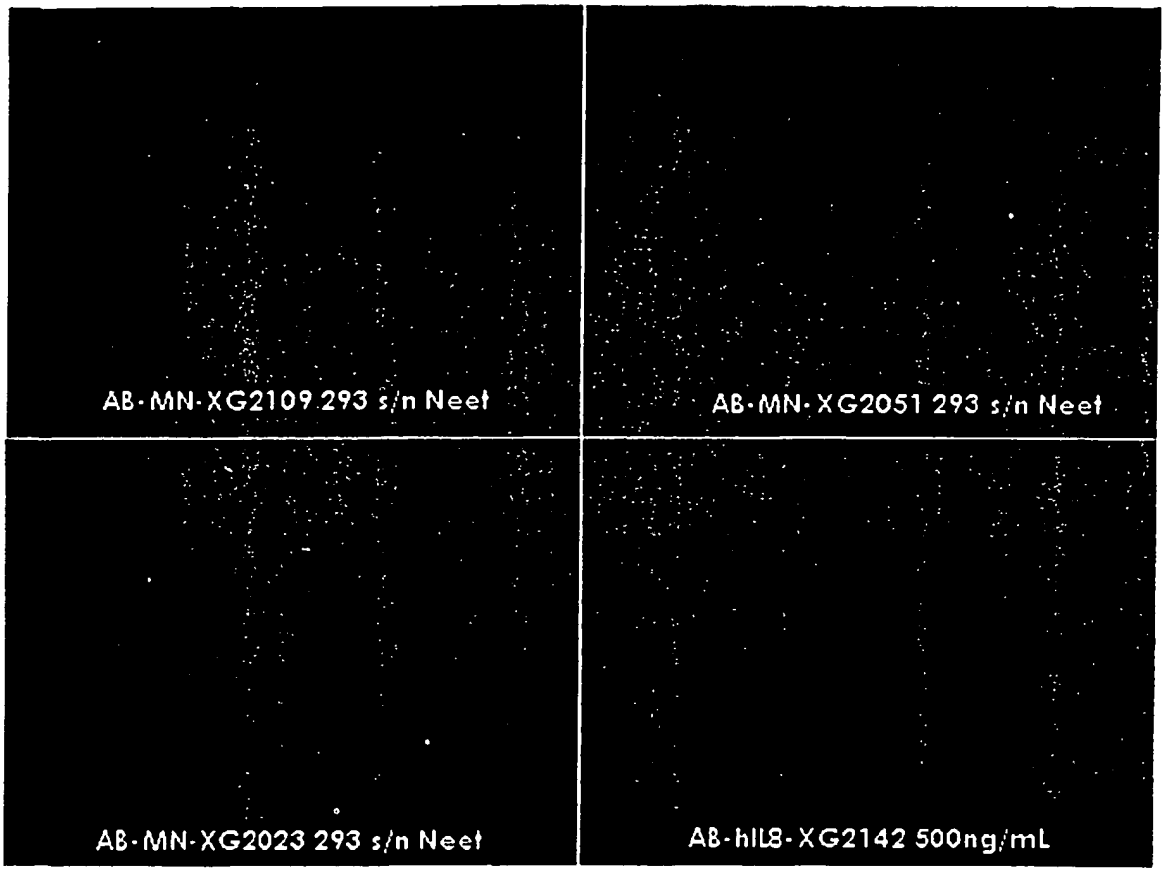

AB-MN-XG2109 293 s/n Neet
AB-MN-XG2051 293 s/n Neet
AB-MN-XG2023 293 s/n Neet
AB-hIL8-XG2142 500ng/mL

FIG. 20

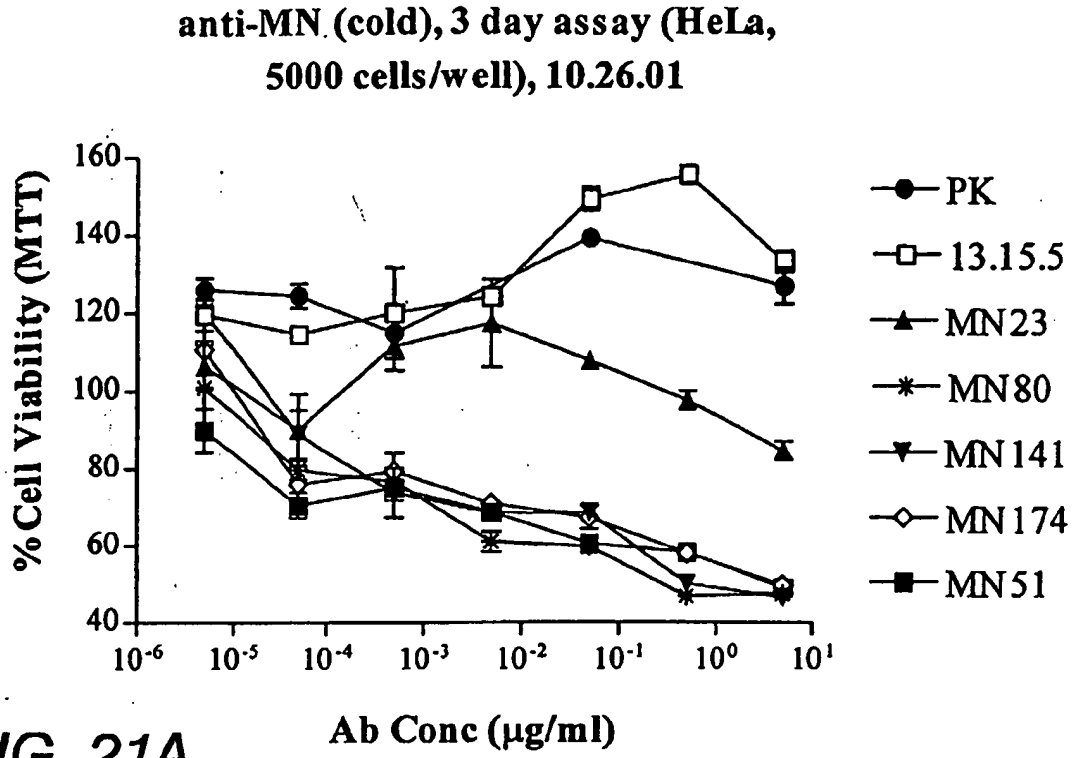


FIG. 21A

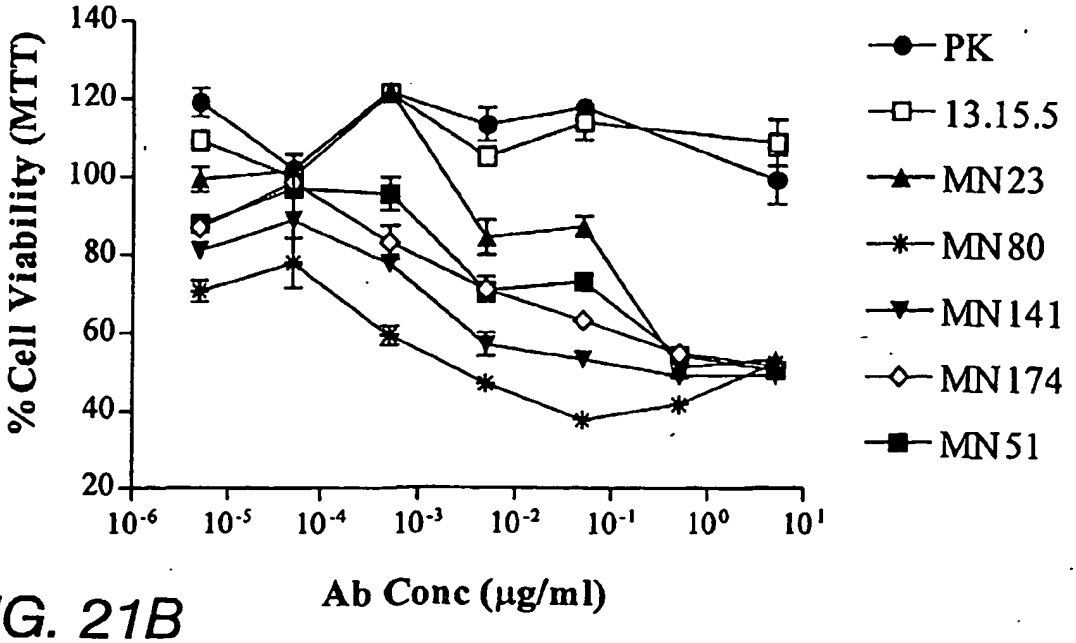


FIG. 21B

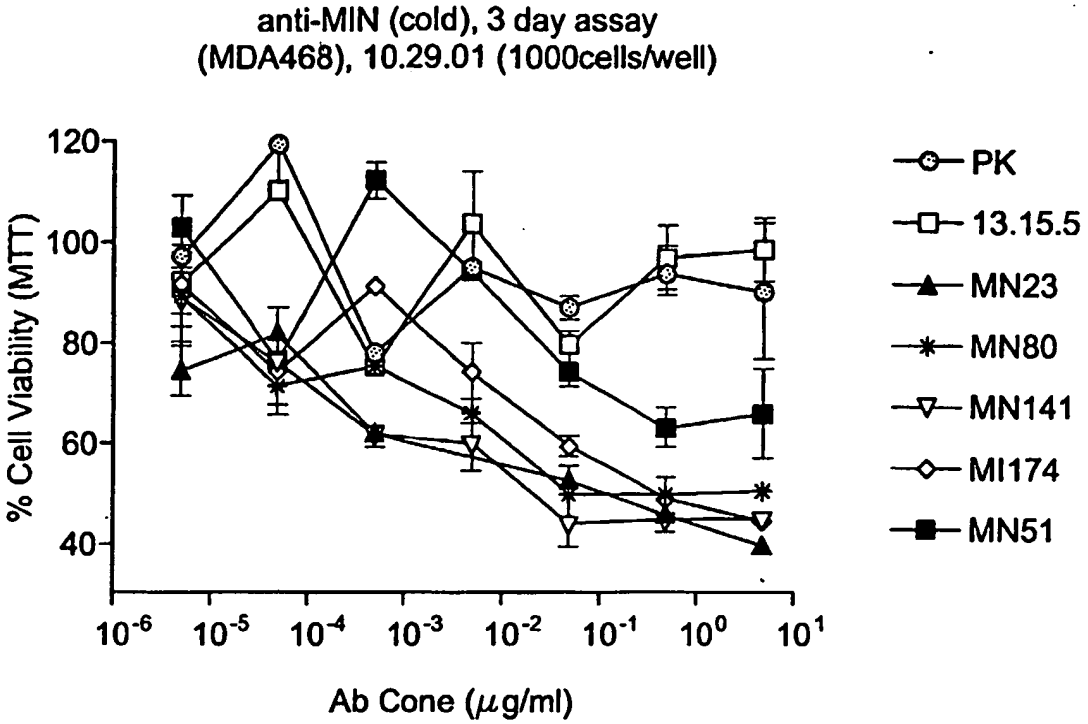
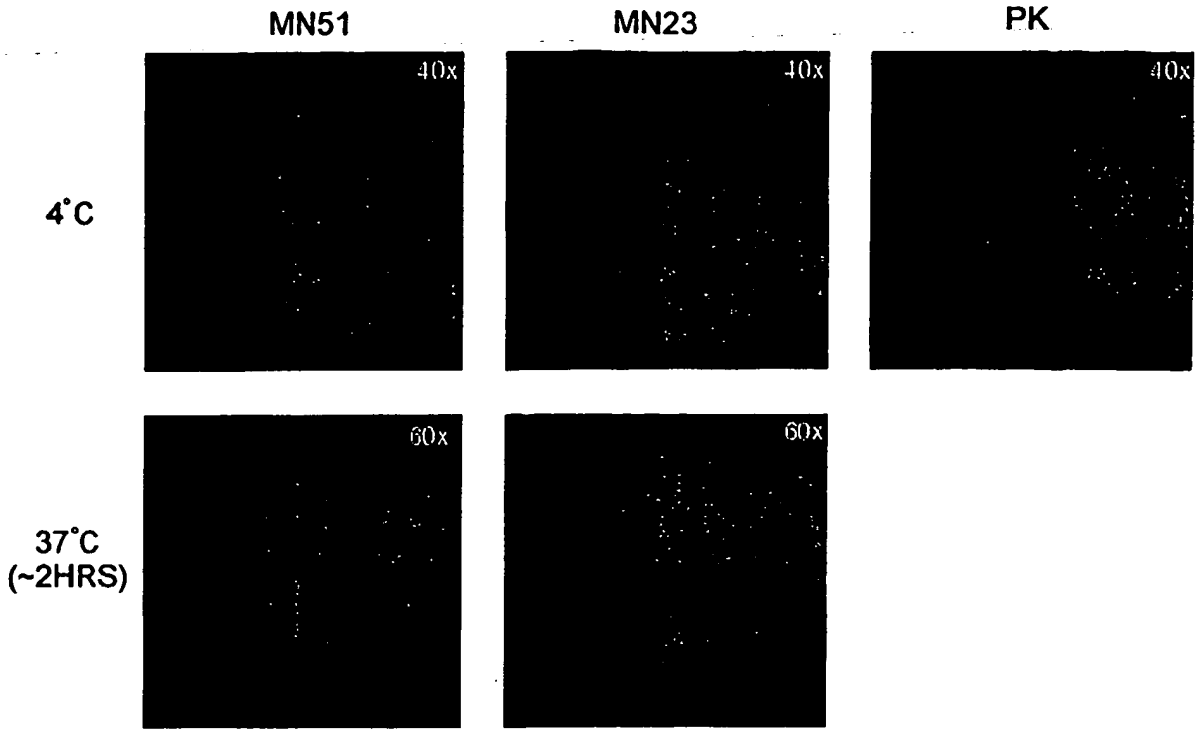

anti-MIN (cold), 3 day assay
(MDA468), 10.29.01 (1000cells/well)
% Cell Viability (MTT)
120
100
80
60
40
10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1}
Ab Cone (μg/ml)
PK
13.15.5
MN23
MN80
MN141
MI174
MN51

FIG. 21C



1°Ab - 5 μg/ml, 60 min @4°C
2°Ab - 1 μl goat anti-human-AF546, red (JIR); 30 min @ 4°C
DNA - Hoechst (1:10,000), blue

FIG. 22

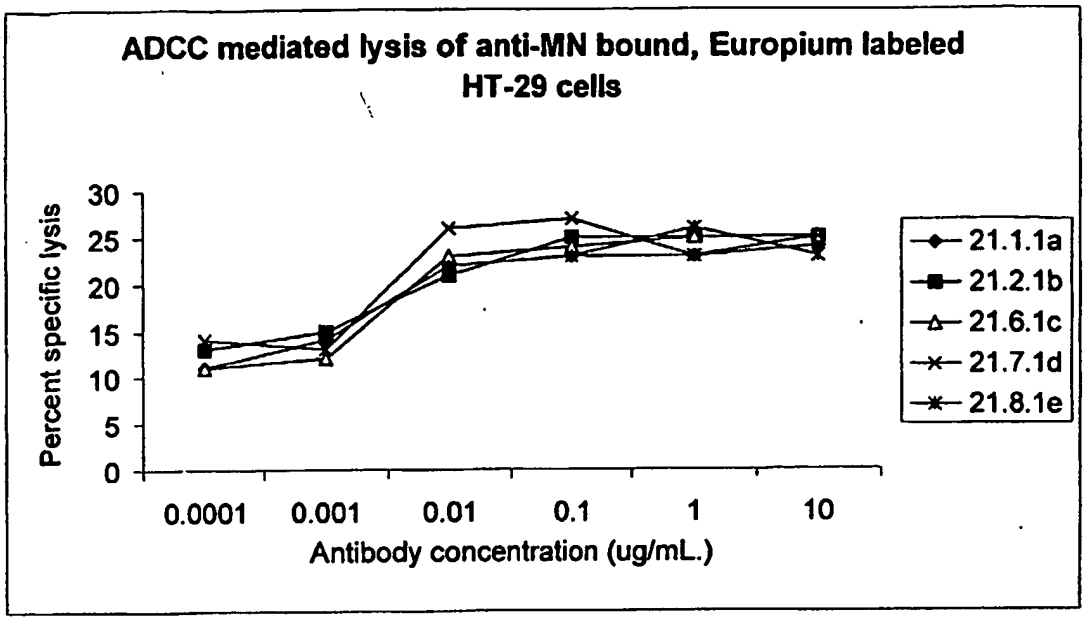


FIG. 23A

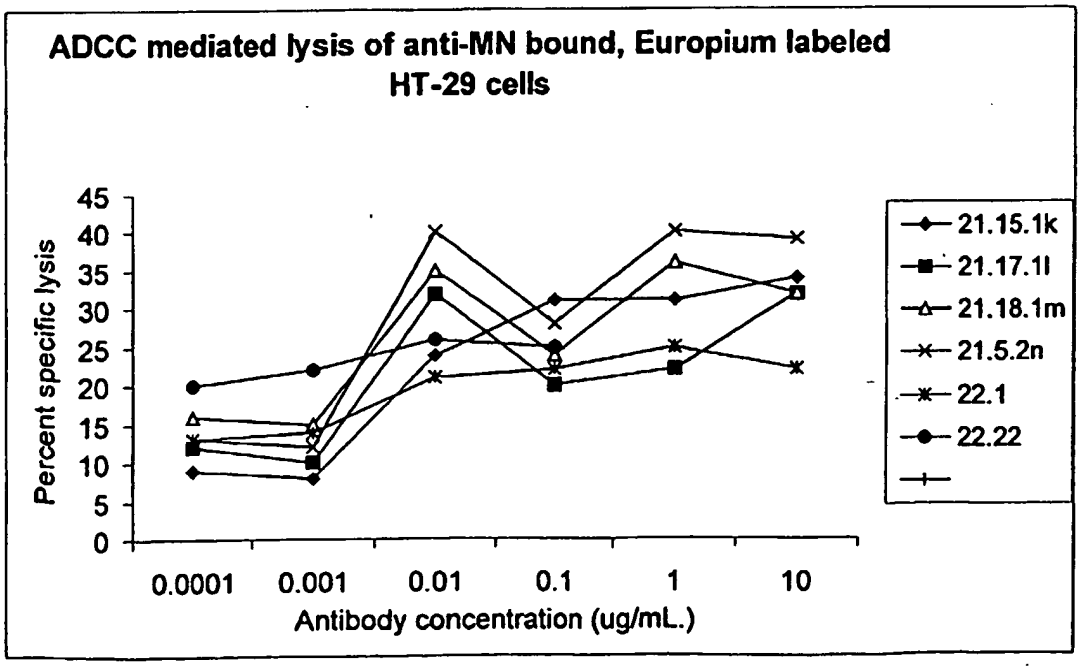


FIG. 23B

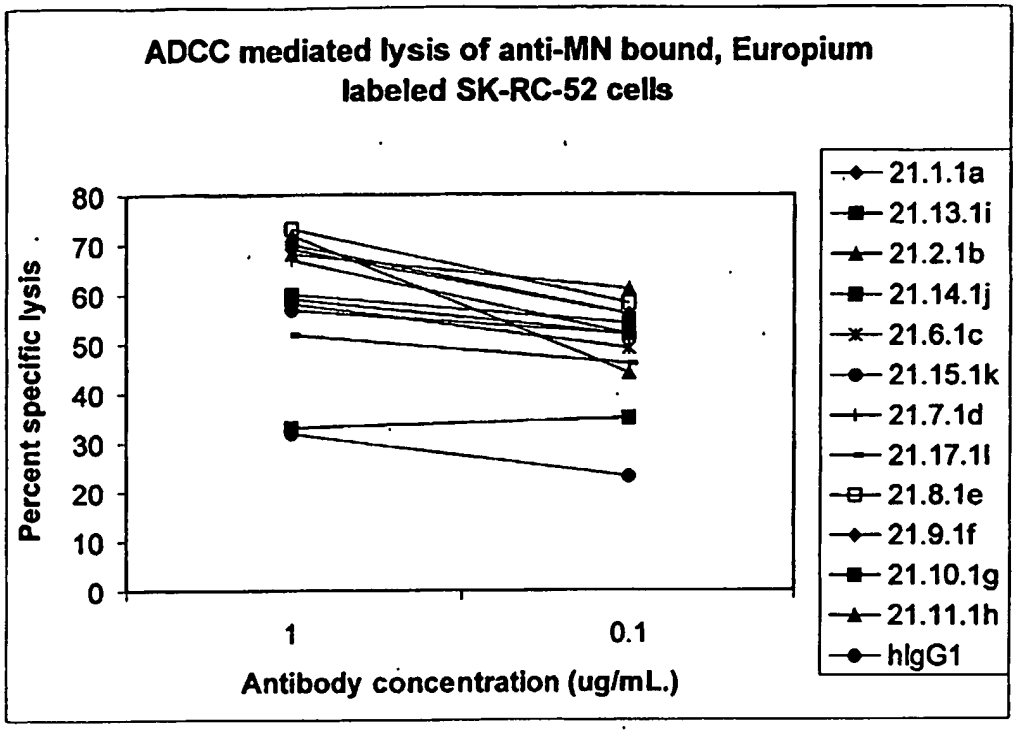

ADCC mediated lysis of anti-MN bound, Europium labeled SK-RC-52 cells
Percent specific lysis
80
70
60
50
40
30
20
10
0
1
0.1
Antibody concentration (ug/mL.)
21.1.1a
21.13.1i
21.2.1b
21.14.1j
21.6.1c
21.15.1k
21.7.1d
21.17.1l
21.8.1e
21.9.1f
21.10.1g
21.11.1h
hIgG1

FIG. 23C

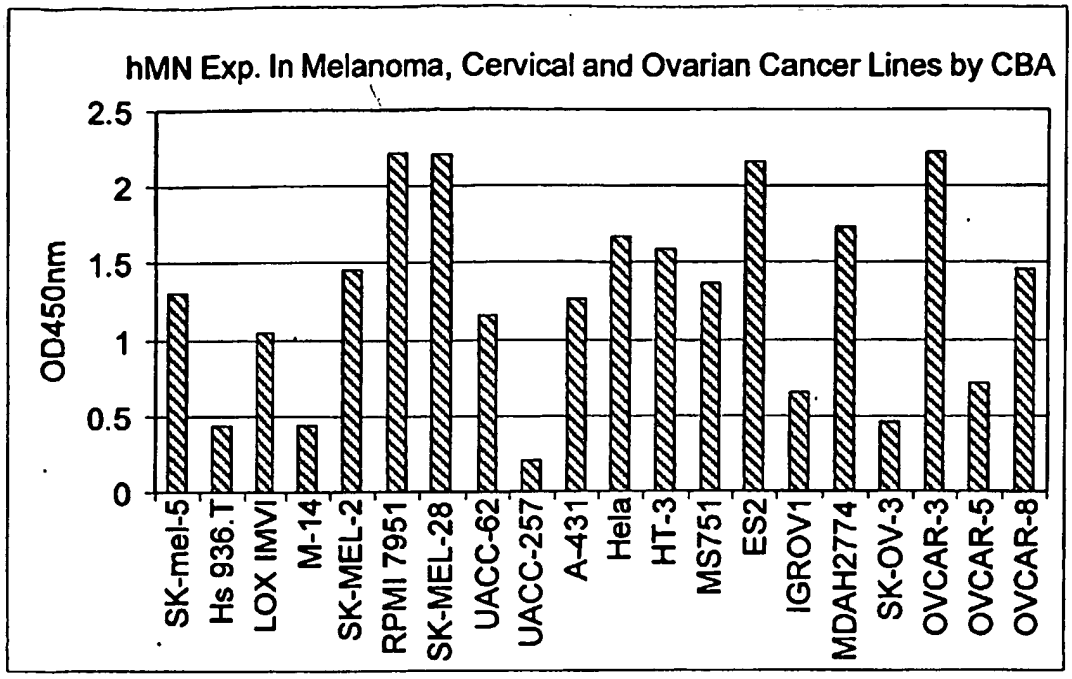


FIG. 24A

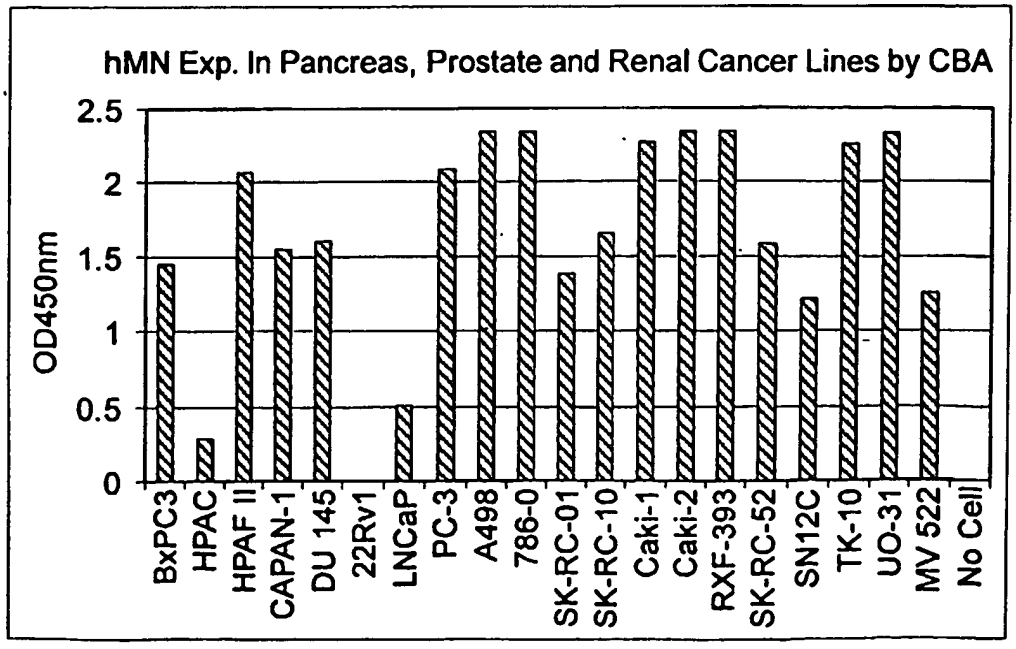


FIG. 24B

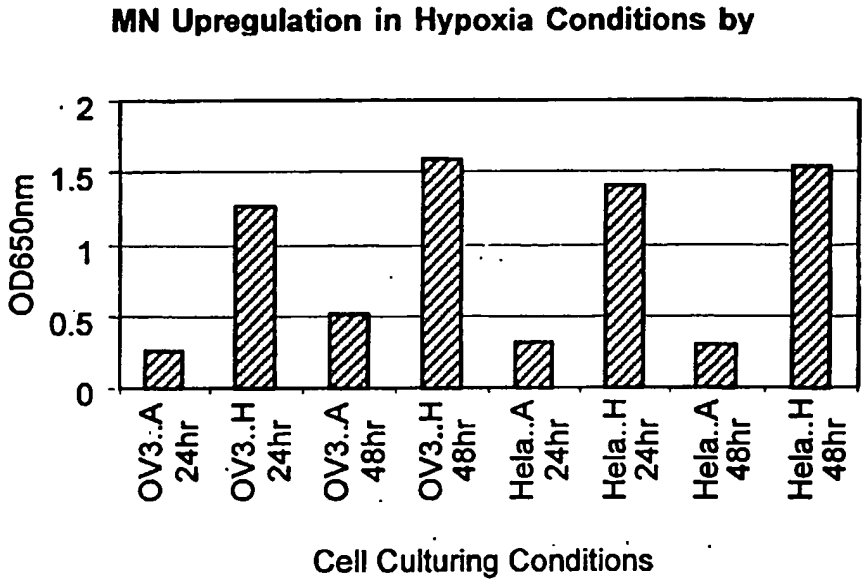

MN Upregulation in Hypoxia Conditions by
OD650nm
2
1.5
1
0.5
0
OV3..A 24hr
OV3..H 24hr
OV3..A 48hr
OV3..H 48hr
Hela..A 24hr
Hela..H 24hr
Hela..A 48hr
Hela..H 48hr
Cell Culturing Conditions

FIG. 25

Chain Name	V	D	J	FR1	CDR1	FR2	CDR2
		Germline		QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTGYYMH	WVRQAPGQGLEWMG	WINPNSGGTNYAQKFQG
AX014H21_8_1N1G2	VH1-2	D7-27	JH6b	----------	----------	----------	----------
		Germline		QVQLQESGPGLVKPSETLSLTCTVS	GGSISSYYWS	WIRQPAGKGLEWIG	RIYTSGSTNYNPSLKS
AX014H22_5N1G2	VH4-4	D3-3	JH6b	----------	-----F---N	----------V-	----------
AX014H22_19N1G2	"	"	"	----------	-----F---N	----------V-	----------
AX014H22_3N1G2	"	"	"	----------	------N---	----------	----------
		Germline		QVQLQESGPGLVKPSETLSLTCTVS	GGSVSSGGYYWS	WIRQPPGKGLEWIG	YIYYSGSTNYNPSLKS
AX014H22_14N1G2	VH4-61	D3-10	JH4b	----------	----I-----	----------	-------S--------
		Germline		EVQLLESGGGLVQPGGSLRLSCAAS	GFTFSSYAMS	WVRQAPGKGLEWVS	AISGSGGSTYYADSVKG
AX014H22_28_1N1G2	VH3-23	D1-26	JH6b	Q---EQ----------	----------	----------	-------G--------
		Germline		QVQLQESGPGLVKPSQTLSLTCTVS	GGSISSGGYYWS	WIRQHPGKGLEWIG	YIYYSGSTYYNPSLKS
AX014H22_23N1G2	VH4-31	D4-17	JH6b	----------	----N-----	----------	----------
AX014H22_9N1G2	"	"	"	----------	----N-----	----------	----------
		Germline		QVQLVESGGGVVQPGRSLRLSCAAS	GFTFSSYGMH	WVRQAPGKGLEWVA	VISYDGSNKYYADSVKG
AX014H22_7N1G2	VH3-30	D3-10	JH6b	----------	----------	----------	----------
		Germline		QVQLQESGPGLVKPSETLSLTCTVS	GGSISSYYWS	WIRQPAGKGLEWIG	RIYTSGSTNYNPSLKS
AX014H21_17_1N1G2	VH4-4	D6-19	JH6b	----------	----------	----------	----------
AX014H22_29N1G2	"	"	"	----------	----------	----------	----------
		Germline		QVQLQQSGPGLVKPSQTLSLTCAIS	GDSVSSNSAAWN	WIRQSPSRGLEWLG	RIYYRSKWYNDYAVSVKS
AX014H22_10N1G2	VH6-1	D1-26	JH4b	----------	----------	----------	----------F------
AX014H22_24N1G2	"	"	"	----------	----------	----------	----------F------
		Germline		QVQLQESGPGLVKPSQTLSLTCTVS	GGSISSGGYYWS	WIRQHPGKGLEWIG	YIYYSGSTYYNPSLKS
AX014H21_5_2N1G2	VH4-31	D3-10	JH4b	----EQ----------	--------R---	----------	----------
AX014H21_6_1N1G2	"	"	"	----------	----------	----------	----------
		Germline		QVQLQESGPGLVKPSQTLSLTCTVS	GGSISSGGYYWS	WIRQHPGKGLEWIG	YIYYSGSTYYNPSLKS
AX014H22_21N1G2	VH4-31	D4-11	JH6b	----------A-----	----------	----------	------Y---------
		Germline		QVQLQESGPGLVKPSQTLSLTCTVS	GGSISSGGYYWS	WIRQHPGKGLEWIG	YIYYSGSTYYNPSLKS
AX014H22_16N1G2	VH4-31	D2-21	JH6b	----------	-------V----	----------	-----R----------
		Germline		QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTGYYMH	WVRQAPGQGLEWMG	WINPNSGGTNYAQKFQG
AX014H22_4N1G2	VH1-2	D1-26	JH4b	----------	----------	----------	----------
		Germline		QVQLQESGPGLVKPSETLSLTCTVS	GGSISSYYWS	WIRQPPGKGLEWIG	YIYYSGSTNYNPSLKS
AX014H21_1_1N1G2	VH4-59	D3-9	JH4b	----------	----------	----------	----------
AX014H22_27N1G2	"	"	"	----------	----------	----------	----------
AX014H21_14_1N1G2	"	"	"	----------	----------	----------	----------
AX014H22_17N1G2	"	"	"	----------	----------	----------	----------
AX014H22_26N1G2	"	"	"	----------	-----RN---	----------	--------K-------

FIG. 26A

Chain Name	V	D	J	FR3	CDR3	J	
		Germline		RVIMTRDTSISTAYMELSRLRSDDTAVYYCAR	##L####YYYYGMDV	WGQGTTVTVSS	SEQ ID NO:124
AX014H21_8_1N1G2	VH1-2	D7-27	JH6b	----------T-	GE-WAEG--------	----------	SEQ ID NO:125
		Germline		RVIMSVDTSKNQFSLKLSSVTAADTAVYYCAR	##FLEW#YYYGMDV	WGQGTTVTVSS	SEQ ID NO:126
AX014H22_5N1G2	VH4-4	D3-3	JH6b	----------	GG----D-------	----------	SEQ ID NO:6
AX014H22_19N1G2	"	"	"	----------	GG----D-------	----------	SEQ ID NO:3
AX014H22_3N1G2	"	"	"	----------	DQG-----PL--	----------	SEQ ID NO.5
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCA#	#MVRG##FDY	WGQGTLVTVSS	SEQ ID NO:127
AX014H22_14N1G2	VH4-61	D3-10	JH4b	-------A-------R----------R	S----VS---	----------	SEQ ID NO:128
		Germline		RFTISRDNSKNTLYLQMNSLRAEDTAVYYC##	G#YYYYYGMDV	WGQGTTVTVSSA	SEQ ID NO:129
AX014H22_28_1N1G2	VH3-23	D1-26	JH6b	----------AS	-R-----D---	----------	SEQ ID NO:130
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	##V##YYYYGMDV	WGQGTTVTVSS	SEQ ID NO:131
AX014H22_23N1G2	VH4-31	D4-17	JH6b	--I------E----------	ER-TD-----L--	----------	SEQ ID NO:18
AX014H22_9N1G2	"	"	"	--I------E----------	ER-TD-----L--	----------	SEQ ID NO:19
		Germline		RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	##ITMVRG###YYGMDV	WGQGTTVTVSS	SEQ ID NO:132
AX014H22_7N1G2	VH3-30	D3-10	JH6b	---F----------	RG------APS------	----------	SEQ ID NO:133
		Germline		RVIMSVDTSKNQFSLKLSSVTAADTAVYYCAR	##QWL##YGMDV	WGQGTTVTVSS	SEQ ID NO:134
AX014H21_17_1N1G2	VH4-4	D6-19	JH6b	----------	DG---ED-----	----------	SEQ ID NO:2
AX014H22_29N1G2	"	"	"	----------	DG---ED-----	----------	SEQ ID NO:4
		Germline		RITINPDTSKNQFSLQLNSVTPEDTAVYYCAR	WELL#FDY	WGQGTLVTVSS	SEQ ID NO:135
AX014H22_10N1G2	VH6-1	D1-26	JH4b	---F----------	----G---	----------	SEQ ID NO:136
AX014H22_24N1G2	"	"	"	---F----------	----G---	----------	SEQ ID NO:137
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	###YYGSGS##DY	WGQGTLVTVSS	SEQ ID NO:138
AX014H21_5_2N1G2	VH4-31	D3-10	JH4b	-----I----------	AGT------YL--	----------	SEQ ID NO:9
AX014H21_6_1N1G2	"	"	"	----------	AGK------YL--	----------	SEQ ID NO:10
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	#TV##YYYYGMDV	WGQGTTVTVSS	SEQ ID NO:139
AX014H22_21N1G2	VH4-31	D4-11	JH6b	----------	E--TD--------	----------	SEQ ID NO:17
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	GN###YYYGMDV	WGQGTTVTVSS	SEQ ID NO:140
AX014H22_16N1G2	VH4-31	D2-21	JH6b	----------	--AHD--F----	----------	SEQ ID NO:15
		Germline		RVIMTRDTSISTAYMELSRLRSDDTAVYYCAR	#GA##FDY	WGQGTLVTVSS	SEQ ID NO:141
AX014H22_4N1G2	VH1-2	D1-26	JH4b	----------F----	L--TS---	----------	SEQ ID NO:142
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	##YDILTGY#YFDY	WGQGTLVTVSS	SEQ ID NO:143
AX014H21_1_1N1G2	VH4-59	D3-9	JH4b	----------	RG-------D----	----------	SEQ ID NO:24
AX014H22_27N1G2	"	"	"	----------	RG--F----D----	----------	SEQ ID NO:29
AX014H21_14_1N1G2	"	"	"	----------	RG--F----D----	----------	SEQ ID NO:25
AX014H22_17N1G2	"	"	"	----------	RG-------D----	----------	SEQ ID NO:27
AX014H22_26N1G2	"	"	"	----------R	S---F-----HV---	----------	SEQ ID NO:28

FIG. 26B

Chain Name	V	D	J	FR3	CDR3	J	
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	DGYNYWYFDL	WGRGTLVTVSS	SEQ ID NO:144
AX014H22_11N1G2	VH4-31	D5-24	JH2	----------	----------	----------	SEQ ID NO:13
		Germline		RFTISRDNAKNSLYLQMNSLRDEDTAVYYCAR	######GMDV	WGQGTTVTVSSA	SEQ ID NO:145
AX014H22_13_1N1G2	VH3-48		JH6b	-------S----------	SLRSG----	----------	SEQ ID NO:146
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	##YYDILTG###YGMDV	WGQGTTVTVSS	SEQ ID NO:147
AX014H22_15N1G2	VH4-31	D3-9	JH6b	----------	DR-------YYN-----	----------	SEQ ID NO:14
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	####FDY	WGQGTLVTVSS	SEQ ID NO:148
AX014H22_25N1G2	VH4-39		JH4b	----------	HGSF---	----------	SEQ ID NO:22
AX014H21_10_1N1G2	"	"	"	----------	HGSF---	----------	SEQ ID NO:21
		Germline		RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR	###LLWFGEL###YGMDV	WGQGTTVTVSS	SEQ ID NO:149
AX014H22_30N1G2	VH3-33	D3-10	JH6b	----------	DRG-------SPH-----	----------	SEQ ID NO:150
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	VLLWFG##YGMDV	WGQGTTVTVSS	SEQ ID NO:151
AX014H21_9_1N1G2	VH4-31	D3-10	JH6b	----------	------ED--V--	----------	SEQ ID NO:12
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCA#	#YYDILTGY##AFDI	WGQGTMVTVSS	SEQ ID NO:152
AX014H22_18N1G2	VH4-31	D3-9	JH3b	----------R	T--------PD----	----------	SEQ ID NO:16
AX014H21_7_1N1G2	"	"	"	-I----------	T---F---YPD----	----------	SEQ ID NO:11
AX014H22_20N1G2	"	"	"	----------R	T-------YPD----	----------	SEQ ID NO:153
AX014H21_2_1N1G2	"	"	"	----------R	T--------PD----	----------	SEQ ID NO:8
		Germline		RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	###SSS#YYYYYGMDV	WGQGTTVTVSSA	SEQ ID NO:154
AX014H22_8_1N1G2	VH4-59	D6-13	JH6b	----------T----------	DQH---V---------	----------	SEQ ID NO:30
AX014H22_12N1G2	"	"	"	----------	DQH---F---------	----------	SEQ ID NO:26

FIG. 27B

Chain Name	V	J	FR1	CDR1	FR2	CDR2
		Germline	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS
AX014H22_15N1K	012	JK4	----------	----------	------V-----	V------
AX014H22_23N1K	"	"	----------	----------	----------	-------
AX014H21_9_1N1K	"	"	----------	-----TN---	----------	-------
AX014H22_16N1K	"	"	----------	-----TN---	------V-----	V------
		Germline	EIVLTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT
AX014H22_3N1K	A27	JK5	----------	-------Y---	----------	-------
		Germline	DIVMTQSPLSLPVTPGEPASISC	RSSQSLLHSNGYNYLD	WYLQKPGQSPQLLIY	LGSNRAS
AX014H21_6_1N1K	A3	JK4	----------	----------	----------	-------
AX014H21_5_2N1K	"	"	----------	----------	------C-----	-------
		Germline	EIVLTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT
AX014H22_8_1N1K	A27	JK4	-TQ-------	-----IYIN---	---------S	-------
AX014H22_27N1K	"	"	----------	W-----F-N---	----------	-------
AX014H22_5N1K	"	"	----------	-------VT---	----------	-------
AX014H21_17_1N1K	"	"	----------	W-----F-N---	----------	-------
AX014H22_11N1K	"	"	----------	-----G----	-------T----	-------
AX014H21_8_1N1K	"	"	----------	----------	----------	-------
AX014H22_19N1K	"	"	----------	-------VT---	----------	-------
AX014H22_4N1K	"	"	----------	----------	----------	-------
		Germline	DIQMTQSPSSLSASVGDRVTITC	RASQGIRNDLG	WYQQKPGKAPKRLIY	AASSLQS
AX014H22_30N1K	A30	JK4	----------	----------	----------	-------
AX014H22_14N1K	"	"	----------	----------	----------	-------
AX014H22_28_1N1K	"	"	--RL------	----------	----------	-------
		Germline	DIQMTQSPSSVSASVGDRVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	AASSLQS
AX014H21_1_1N1K	L5	JK5	----------	-------I--T	----------	-------
AX014H22_29N1K	"	"	----------	----------	-F--------	-------
AX014H21_14_1N1K	"	"	----------	----------	-F--------	-------
AX014H22_17N1K	"	"	----------	-------I--T	----------	-------
		Germline	DIQMTQSPSSLSASVGDRVTITC	RASQGIRNDLG	WYQQKPGKAPKRLIY	AASSLQS
AX014H22_24N1K	A30	JK3	----------	----------	----------	-T-N---
AX014H22_10N1K	"	"	----------	-------Y---	----------	-T-N---
		Germline	DVVMTQSPLSLPVTLGQPASISC	RSSQSLVYSDGNTYLN	WFQQRPGQSPRRLIY	KVSNWDS
AX014H21_10_1N1K	A1	JK4	----------	-------D----	----------	-------
		Germline	EIVLTQSPGTLSLSPGERATLSC	RASQSVSSSYLA	WYQQKPGQAPRLLIY	GASSRAT
AX014H22_9N1K	A27	JK2	----------	-----Y-N-F-	----------	------A
AX014H22_26N1K	"	"	----------	-----Y-N-F-	----------	------A

FIG. 28A

Chain Name	V	J	FR3	CDR3	J	
		Germline	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQSYST##T	FGGGTKVEIK	SEQ ID NO:155
AX014H22_15N1K	012	JK4	----------	------PL-	----------	SEQ ID NO:67
AX014H22_23N1K	"	"	----------	----I----	-----E----	SEQ ID NO:69
AX014H21_9_1N1K	"	"	----------	----I----	-----E----	SEQ ID NO:66
AX014H22_16N1K	"	"	----------	------PL-	----------	SEQ ID NO:68
		Germline	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSPIT	FGQGTRLEIK	SEQ ID NO:156
AX014H22_3N1K	A27	JK5	----------	----T----	----------	SEQ ID NO:43
		Germline	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQALQT##T	FGGGTKVEIK	SEQ ID NO:157
AX014H21_6_1N1K	A3	JK4	----------	------PL-	----------	SEQ ID NO:33
AX014H21_5_2N1K	"	"	----------	------PL-	----------	SEQ ID NO:32
		Germline	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSLT	FGGGTKVEIKR	SEQ ID NO:80
AX014H22_8_1N1K	A27	JK4	----------	----R---	-----E-----	SEQ ID NO:36
AX014H22_27N1K	"	"	----------P----------	------PL-	----------	SEQ ID NO:42
AX014H22_5N1K	"	"	----------	-----SV-	----------	SEQ ID NO:45
AX014H21_17_1N1K	"	"	----------P----------	------PL-	----------	SEQ ID NO:37
AX014H22_11N1K	"	"	----------	----F----	----------	SEQ ID NO:39
AX0114H21_8_1N1K	"	"	----------S-	--------	----------	SEQ ID NO:38
AX014H22_19N1K	"	"	----------	-----SV-	----------	SEQ ID NO:40
AX014H22_4N1K	"	"	----------S-	--------	----------	SEQ ID NO:44
		Germline	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	LQHNSY##T	FGGGTKVEIK	SEQ ID NO:158
AX014H22_30N1K	A30	JK4	----------D----------	---Y--PL-	----------	SEQ ID NO:58
AX014H22_14N1K	"	"	----------	------PL-	----------	SEQ ID NO:51
AX014H22_28_1N1K	"	"	----------	------PL-	----------	SEQ ID NO:57
		Germline	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFPIT	FGQGTRLEIK	SEQ ID NO:159
AX014H21_1_1N1K	L5	JK5	----------	---------	----------	SEQ ID NO:60
AX014H22_29N1K	"	"	----------	---------	----------	SEQ ID NO:64
AX014H21_14_1N1K	"	"	----------	---------	----------	SEQ ID NO:61
AX014H22_17N1K	"	"	----------	---------	----------	SEQ ID NO:63
		Germline	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	LQHNSYPPT	FGPGTKVDIK	SEQ ID NO:160
AX014H22_24N1K	A30	JK3	----------	---------	----------	SEQ ID NO:55
AX014H22_10N1K	"	"	----------	---------	----------	SEQ ID NO:50
		Germline	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQGTHWPPLT	FGGGTKVEIKR	SEQ ID NO:161
AX014H21_10_1N1K	A1	JK4	----------	----------	----------	SEQ ID NO:162
		Germline	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQYGSSP###	FGQGTKLEIK	SEQ ID NO:163
AX014H22_9N1K	A27	JK2	----------	----T--RFS	----------	SEQ ID NO:46
AX014H22_26N1K	"	"	----------	----T--RFS	----------	SEQ ID NO:41

FIG. 28B

Chain Name	V	J	FR1	CDR1	FR2	CDR2
		Germline	DIQMTQSPSSVSASVGDRVTITC	RASQGISSWLA	WYQQKPGKAPKLLIY	AASSLQS
AXO14H22_12N1K	L5	JK3	----------	----------	----------	-------
		Germline	DIVMTQSPLSLPVTPGEPASISC	RSSQSLLHSNGYNYLD	WYLQKPGQSPQLLIY	LGSNRAS
AX014H22_7N1K	A3	JK2	----------	-------Y--------	----------F---	-------
		Germline	DIQMTQSPSSLSASVGDRVTITC	RASQGIRNDLG	WYQQKPGKAPKRLIY	AASSLQS
AX014H22_18N1K	A30	JK1	----------	----D------	----------C	-------
AX014H21_7_1N1K	"	"	----------	----A------	----------	-------
AX014H22_21N1K	"	"	----------	----A------	----------	-------
AX014H22_20N1K	"	"	----------	----------	----------	-------
AX014H22_25N1K	"	"	----------	----------	----------	-------
AX014H21_2_1N1K	"	"	----------	----D------	----------C	-------
		Germline	DIVMTQTPLSSPVTLGQPASISC	RSSQSLVHSDGNTYLS	WLQQRPGQPPRLLIY	KISNRFS
AX014H22_13_1N1K	A23	JK4	----------	-A----------	----------	-------

FIG. 29A

Chain Name	V	J	FR3	CDR3	J	
		Germline	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQANSFPLT	FGPGTKVDIK	SEQ ID NO:164
AX014H22_12N1K	L5	JK3	----------	---------	----------	SEQ ID NO:62
		Germline	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	MQALQTP##	FGQGTKLEIK	SEQ ID NO:165
AX014H22_7N1K	A3	JK2	----------	-------RS	----------	SEQ ID NO:34
		Germline	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	LQHNSYPWT	FGQGTKVEIK	SEQ ID NO:166
AX014H22_18N1K	A30	JK1	----S----------	---------	----------	SEQ ID NO:52
AX014H21_7_1N1K	"	"	----------S----------	--YH-----	----------	SEQ ID NO:49
AX014H22_21N1K	"	"	----------S----------	--YH-----	----------	SEQ ID NO:54
AX014H22_20N1K	"	"	----------T----	--YK-----	----------	SEQ ID NO:53
AX014H22_25N1K	"	"	----------T----	--YK-----	----------	SEQ ID NO:56
AX014H21_2_1N1K	"	"	----S----------	---------	----------	SEQ ID NO:48
		Germline	GVPDRFSGSGAGTDFTLKISRVEAEDVGVYYC	MQATQF##T	FGGGTKVEIKR	SEQ ID NO:167
AX014H22_13_1N1K	A23	JK4	----------	------PL-	----------	SEQ ID NO:168

FIG. 29B